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ABSTRACT

This course, the third of three courses in the medical laboratory technician field adapted from military curriculum materials for use in vocational and technical education, was designed as a refresher course for student self-study and evaluation. It is suitable for use by advanced students or beginning students participating in a supervised laboratory or on-the-job learning situation. The course is divided into three volumes containing student workbooks, readings, and tests. Volume 1 covers blood composition and functions, blood counts, ertrocytes, leukocytes, and coagulation. Volume 2 presents information concerning blood banking; this information includes immunohematology, blood group systems, transfusion of blood, and the operation of a blood center. The final volume discusses the principles of serology, the agglutination test, the fixation and precipition test, and the serological test for syphilis. A glossary of technical terms used in the three volumes is printed at the back of volume 3. Each of the volumes contains chapters with objectives, text, review exercises, and answers to the exercises. A volume review exercise (without answers) is provided. (KC)

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The military-developed curriculum materials in this course package were selected by the National Center for Research in Vocational Education Military Curriculum Project for dissemination to the six regional Curriculum Coordination Centers and other instructional materials agencies. The purpose of disseminating these courses was to make curriculum materials developed by the military more accessible to vocational educators in the civilian setting.

The course materials were acquired, evaluated by project staff and practitioners in the field, and prepared for dissemination. Materials which were specific to the military were deleted, copyrighted materials were either omitted or approval for their use was obtained. These course packages contain curriculum resource materials which can be adapted to support vocational instruction and curriculum development.

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Military Curriculum Materials Dissemination Is . . .

an activity to increase the accessibility of military-developed curriculum materials to vocational and technical educators.

This project, funded by the U.S. Office of Education, includes the identification and acquisition of curriculum materials in print form from the Coast Guard, Air Force, Army, Marine Corps and Navy.

Access to military curriculum materials is provided through a "Joint Memorandum of Understanding" between the U.S. Office of Education and the Department of Defense.

The acquired materials are reviewed by staff and subject matter specialists, and courses deemed applicable to vocational and technical education are selected for dissemination.

The National Center for Research in Vocational Education is the U.S. Office of Education's designated representative to acquire the materials and conduct the project activities.

Project Staff:

Wesley E. Budke, Ph.D., Director
National Center Clearinghouse

Shirley A. Chase, Ph.D.
Project Director

What Materials Are Available?

One hundred twenty courses on microfiche (thirteen in paper form) and descriptions of each have been provided to the vocational Curriculum Coordination Centers and other instructional materials agencies for dissemination.

Course materials include programmed instruction, curriculum outlines, instructor guides, student workbooks and technical manuals.

The 120 courses represent the following sixteen vocational subject areas:

- Agriculture
- Aviation
- Building & Construction Trades
- Clerical Occupations
- Communications
- Drafting
- Electronics
- Engine Mechanics
- Food Service
- Health
- Heating & Air Conditioning
- Machine Shop
- Management & Supervision
- Meteorology & Navigation
- Photography
- Public Service

The number of courses and the subject areas represented will expand as additional materials with application to vocational and technical education are identified and selected for dissemination.

How Can These Materials Be Obtained?

Contact the Curriculum Coordination Center in your region for information on obtaining materials (e.g., availability and cost). They will respond to your request directly or refer you to an instructional materials agency closer to you.

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MEDICAL LABORATORY TECHNICIAN - HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

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MEDICAL LABORATORY TECHNICIAN-HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

Correspondence Course

10-4

Developed by:

United States Air Force

Development and Review Dates:

Unknown

Occupational Area:

Health

Cost:

Print Pages:

282

Availability:

Military Curriculum Project, The Center for Vocational Education, 1960 Kenny Rd., Columbus, OH 43210

Suggested Background:

Chemistry, biology, zoology, *Medical Laboratory-Clinical Chemistry and Urinalysis*, (10-2) and *Microbiology*, (10-3)

Target Audiences:

Grades 10-adult

Organization of Materials:

Student workbooks containing objectives, assignments, chapter review exercises and answers, and volume review exercises; text

Type of Instruction:

Individualized, self-paced

Type of Materials:			**No. of Pages:**	**Average Completion Time:**
Volume 1	–	*Hematology*	55	Flexible
		Workbook	41	
Volume 2	–	*Laboratory Procedures in Blood Banking and Immunohematology*	43	Flexible
		Workbook	31	
Volume 3	–	*Serology*	54	Flexible
		Workbook	36	

Supplementary Materials Required:

None

CCC 9

Expires July 1, 1978

Course Description

This course is the third of three courses in the Medical Laboratory Technician field to upgrade the Specialist (skilled) worker to the Technician (advanced) level. The course contains basic information and could be used as a refresher course, but it is designed to be used by advanced students, or beginning students in a supervised laboratory or on-the-job learning situation. The first course, *Medical Laboratory Technician-Clinical Chemistry and Urinalysis,* and the second course, *Microbiology,* are prerequisites to this course. The duties of a Medical Technician/Specialist are:

- Performs hematological tests
- Performs urinalysis
- Performs chemical analysis
- Assists in blood bank duties
- Performs microbiological and serological tests
- Accomplishes general medical laboratory duties
- Supervises medical laboratory personnel

This course is divided into three volumes containing student workbooks, readings, and tests. One section on blood clotting was deleted because it contained copyright material and other sections were deleted because they dealt with specific military administrative procedures.

Volume 1	–	*Hematology* discusses the physiology of blood, the complete blood count and related studies, erythrocyte studies, leukocyte and thrombocyte maturation, and blood coagulation studies. The section discussing the principles coagulation was deleted for copyright reasons, however the section on tests for coagulation deficiencies remains.
Volume 2	–	*Laboratory Procedures in Blood Banking and Immunohematology* discusses immunohematology, blood group systems, blood for transfusion, and the blood donor center. One section dealing with the Military Blood Donor System was deleted because of references to specific military forms and procedures.
Volume 3	–	*Serology* explains the principles of serology, agglutination tests, latex-fixation, precipitin, and ASO tests, and serological tests for syphilis. The final chapter on medical laboratory administration was deleted because of its reference to specific military procedures and forms.

Each of the volumes contains chapters with objectives, text, review exercises and answers to the exercises. A volume review exercise is provided but no answers are available. This course was designed for student self-study and evaluation within the context of a laboratory on-the-job learning situation. The material is useful for beginning students with a good science and math background or workers who wish to upgrade or refresh their skills. Much of the material is review of basic procedures with some supervisory information.

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MEDICAL LABORATORY TECHNICIAN–HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

(AFSC 90470)

Volume 1

Hematology

10-4

Extension Course Institute

Air University

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Preface

THIS COURSE, the final one in the Medical Laboratory Technician series, is made up of three volumes. These three volumes cover hematology, blood banking, and serology. Volume 1 covers blood composition and functions, blood counts, erythrocytes, leukocytes, and coagulation. Volume 2 presents information concerning blood banking. This information includes immunohematology, blood group systems, transfusion-blood, and the operation of a blood donor center. Volume 3 discusses the principles of serology, agglutination test, fixation and precipitin test, and serological test for syphilis. The last chapter of volume 3 is devoted to administrative procedures which are peculiar to the medical laboratory.

A glossary of technical terms used in Volumes 1, 2, and 3 of this CDC is printed at the back of Volume 3.

Included as a separate inclosure to this volume are two foldouts. Whenever you are referred to one of these foldouts in the text, please turn to the inclosure and locate it.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to Med. Svc. Sch. (MSSTW/120), Sheppard AFB, Texas 76311.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Study Reference Guides, Chapter Review Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFB, Alabama 36114, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 36 hours (12 points).

Material in this volume is technically accurate, adequate, and current as of December 1969.

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Acknowledgment

Grateful acknowledgement is made for permission to use copyright material from the article "Abnormal Coagulation States," published in the *Ortho Diagnostic Reporter*, Volume 2, Number 3. Section 13, Principles of Coagulation, has been adapted from this article. Figure 10 is also taken from this article.

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The Physiology of Blood

LIKE THE STUDY OF many other areas of medical science, the study of blood began in the 17th century. Using a microscope of his own design which magnified 160 to 270 times, the Dutch microscopist, Van Leeuwenhoek, described blood cells in 1673. It was the Italian investigator, Marcello Malpighi (1628–1694), who was probably the first to observe blood cells 10 years earlier. Although Harvey is generally credited with discovering the phenomenon of blood circulation in 1628, Malpighi was the first to observe the passage of blood from the arteries into the veins with a microscope, which at most magnified 145 times. (Compare this with your own oil immersion lens which magnifies at nearly 1,000 times when used with a 10 power ocular.) A series of investigations continued and were highlighted in the following century when a role for the red blood cell was suggested. It was not until 1867, however, that hemoglobin and its functions were described. From such observations of the physical characteristics of blood there developed the science of hematology. The word *hematology* is derived from the Greek words, *aima,* meaning blood, and *logos* meaning study.

2. In this chapter we will review the composition and functions of blood. Blood is a tissue in which the cells are suspended in a liquid medium. In this respect, it differs from other tissues of the body which are also groups of specialized cells identified with a common function. The prime function of the blood is to maintain oxygen and food supply for the body cells and prevent accumulation of waste products. However, these are by no means the only functions of blood. Immunologic mechanisms, as well as other physical and chemical activities, involve the blood in many ways.

3. Since blood has a variety of complex functions, the composition of blood must also be complex. The average circulating total blood volume in a 150-pound male is about 3 liters. Formed elements account for nearly 45 percent of the blood volume and 90 percent of the remaining 55 percent is water, but the portion of liquid which is not water includes an uncalculated number of organic and inorganic materials.

1. The Composition of Blood

1-1. Our study of blood components will include both the cells and the medium in which they are suspended. We will consider the functions of blood in terms of the cellular and noncellular components. However, remember that separating the cells from the medium in which they are suspended is a privilege reserved for the laboratory technician. It is not realistic from a biological standpoint to completely separate these functions.

1-2. **Cellular Constituents.** The well known cellular components of blood are, of course, the erythrocytes (RBCs) and the leukocytes (WBCs), as well as the platelets or thrombocytes. Where do these cellular elements come from and what are their functions? First, let's look at the RBCs.

1-3. *Erythrocytes.* The RBC is a living, metabolically active cell and not merely a small globule of protein which transports hemoglobin.

1-4. RBC development (erythropoiesis) is as follows: Primitive blood cells begin to form from specific tissue of the yolk sac early in the development of a human embryo. Following this period of development, the hepatic phase of blood cell development begins at about the second month of fetal life. Erythroblasts appear in the circulation as a result of development from mesenchyme cells in the liver tissue.

1-5. After erythropoiesis begins in the liver, the spleen produces erythrocytes to the end of the fourth month. The thymus is also active in producing blood cells, including RBCs, during this period of hepatic erythropoiesis.

1-6. During the last period of blood formation (hemopoiesis), the red bone marrow takes over the production of all blood cells. This period begins at about the fifth month. Unless the body is placed under some type of stress, the bone marrow alone produces both red and white blood cells.

The primitive cells which gave rise to blood cells in fetal life revert to a "stand by" status in the liver, spleen, lymph nodes, and other parts of the body where they make up the socalled reticuloendothelial (RE) system. The RE system consists of a variety of different types of cells (histiocytes, Kupffer's cells, and others) whose function is to phagocytize particles such as bacteria or worn out cells.

1-7. In the human being, the bone marrow normally produces red blood cells after birth. We have seen that erythropoietic activity in the fetus is quite different. Since it is generally the postnatal activity of erythropoiesis which is of greatest concern to the hematologist and to us, we should be interested in knowing how RBCs are produced in the marrow.

1-8. After birth, red blood cells are produced in the red marrow of the spongy bones. In the adult, this is restricted to the ribs, sternum, vertebrae, certain skull bones, and the epiphyses (ends) of the femur and humerus. This red marrow is in contrast to the yellow marrow which is contained in the diaphysis (shaft) of long bones and has no erythropoietic activity. The red blood cells arise by cell division from *stem cells* in the red marrow. Stem cells are, by definition, undifferentiated cells which maintain their own numbers, but also give rise to other types of cells. Current theories hold that stem cells undergo two different kinds of division, the first to produce their own kind, and the second to produce at least one other type of cell. The stem cells may also produce its own kind of cell in this second type of division. The maturation sequence of erythrocytes is discussed in Chapter 3 of this volume.

1-9. It would be well to mention at this point that cell division in which the nucleus divides without a reduction in the number of chromosomes is referred to as *mitosis*. Blood cells are produced by mitosis of stem cells. This is unlike the reduction division *(meiosis)* which produces sex cells; i.e., the sperm and the egg, in which there is a reduction in the number of chromosomes.

1-10. *Leukocytes.* WBCs of the types we find in adults are rarely found in the early stages of fetal blood formation. Leukocyte production is generally believed to begin during the third or fourth month in the liver and continue to be produced there until a few weeks prior to birth. During this time the thymus, lymph nodes, and spleen produce myelocytes and lymphocytes. After birth, lymphatic tissue plays a role in lymphocyte production, but the primary production is thought to occur in the bone marrow, i.e., *medullary*. Myelocytes and monocytes are definitely medullary in origin after birth. Researchers frequently distinguish between a small race of lymphocytes and larger lymphocytes, with respect to life span, origin, and in some instances, with respect to function. We do not feel that this distinction is of any special importance to the medical laboratory technician. We will consider the maturation sequence of leukocytes in Chapter 4 of this volume. We generally divide leukocytes into granulocytes (neutrophils, eosinophils, and basophils) and agranulocytes (monocytes and lymphocytes).

1-11. *Thrombocytes (platelets).* Fragmented from the cytoplasm of megakaryocytes, thrombocytes vary considerably in size and shape. The cytoplasm contains a number of basophilic (purple) granules which can be observed on Wright stain smears. There are normally four to six thrombocytes per oil immersion field in areas of the slide where they are evenly dispersed. The normal platelet count as determined by phase microscopy is from approximately 45,000 to 375,000 per cubic mm.

1-12. **Control of Cell Production.** There is mounting evidence that hemopoiesis is at least in part under endocrine control. This is suggested by the fact that certain hormone imbalances are accompanied by changes in the blood picture. We will not attempt to discuss even the major hormones and their specific influences. You should merely be aware that erythropoiesis is indeed complex. In addition to hormonal control, there is evidence of neurogenic influence which may possibly be independent of hormone activity. One such controlling substance mentioned widely in the literature is *erythropoietin*. Although little is known about erythropoietin, it is thought to be the most important controlling agent in red cell production. An earlier term, *hemopoietin*, has essentially been dropped from usage.

1-13. The control of leukocyte production is not as well understood as erythropoiesis. One reason for this is that red cells can be conveniently labeled by radioactive means and effectively traced Further, the breakdown products of WBCs are not as readily observable, and RBCs do not continually move in and out of the blood as WBCs do

1-14. The number of circulating cells, either white or red, is the net result of blood production minus destruction and blood loss. We have already mentioned some of the factors which control erythropoiesis. But how are "worn out" cells disposed of? The normal RBC has been shown to have a *half-life* of 28 to 30 days when tagged with radioactive chronium (Cr^{51})[1]. By half-life is meant the time required for one-half the number of erythrocytes tagged to disappear. Other methods yield somewhat different results. The average time of erythrocyte survival from the time the cells enter the peripheral blood until destruction is usually given as 120 days as determined by other methods. Increased destruction of erythrocytes is characteristic of hemolytic diseases. We will discuss erythrocyte abnormalities in greater detail in Chapter 3. Erythrocytes are normally disposed of by the reticuloendothelial system, particularly by the spleen. Hemoglobin that is released in red cell destruction is the source for bile pigments. Iron from blood destruction is used again in the production of new red blood cells.

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[1] *Laboratory Medicine Hematology*, 3rd Ed., p. 687. Miale, John B. C. V. Mosby Co., St. Louis, Missouri, 1967.

1-15. Leukocytes enter and leave the peripheral blood throughout their life span. Consequently, the longevity of a leukocyte has not been worked out with certainty. In fact the fate of lymphocytes has not even been determined.

1-16. **Plasma.** That portion of the blood which remains after the cells have been removed is, of course, plasma. From your study of clinical chemistry you will recall that plasma contains 3.6 to 5.6 grams of albumin per 100 ml. and 1.3 to 3.2 grams of globulin per 100 ml. Plasma also contains fibrinogen, which is not present in serum. The level of fibrinogen in normal human plasma is 0.15 to 0.30 grams per 100 ml. There are many other protein substances present, such as the coagulation factors.

1-17. In addition to proteins, plasma contains certain metabolic components required by cells. You are thoroughly familiar with many of these substances which are measured in the clinical laboratory. Glucose is probably the single most important food substance found in blood. Various ions present in plasma can also be considered nutrients for body cells as well as blood cells. All products of cells may also be found in the blood. In this category we find enzymes, antibodies, and hormones. Cellular waste products include urea, uric acid, and like compounds. In addition to dissolved solids, we find dissolved and combined gases. Foremost among these are oxygen, carbon dioxide, and nitrogen.

2. Functions of Blood

2-1. In section 1 we identified the major components of blood. However, description and taxonomy alone are not the ultimate in a science. Each component of blood is uniquely capable of performing one or more functions. As you read this section, you will very likely be surprised to learn that much is yet unknown about the constituents with which you are so familiar in the clinical laboratory.

2-2. Perhaps the best way to generally describe the functions of blood is to say that blood helps provide a relatively stable internal environment of the body by a variety of mechanisms. Unlike unicellular organisms, body cells are highly specialized and cannot survive independently. They must be supplied and maintained. This is the main theme in the study of blood function. We describe the maintenance of relative biological constancy or integrity as *homeostasis*.

2-3. In practicing hematology in the laboratory, we are concerned more with measurable evidence of dysfunction than with the final result of blood functions. We observe blood cells, measure chemical constituents, and perform serological tests to identify problem areas. This is the duty of a laboratory technician. We seldom reflect on the many complicated tasks the blood must perform. Let's list some of these functions.

- Transport oxygen and carbon dioxide.
- Contribute to the fluid medium which bathes body cells.
- Regulate body temperature by circulation.
- Distribute basic chemical substances, hormones, vitamins, and other metabolites.
- Carry waste products from the cells to the excretory organs.
- Maintain osmotic equilibrium and acid-base balance to provide a suitable cellular medium.

2-4. You will note that we are describing functions of the blood with reference to other body tissues which the blood supports. We are not at this point especially concerned with the ways in which the blood maintains itself. If its only function were to sustain itself, there would be no justification for its existence. Yet, as technicians, we frequently think of blood dyscrasias as independent difficulties with the blood rather than with their effect on other organs of the body. This is because we are not involved in the clinical practice of medicine, but with scientific measurement of biological entities. As you progress to the 7 level, however, you must discern relationships as well as report single items of laboratory data. Please realize that you are not being asked to reach clinical conclusions, but to appreciate the involved interrelationships which exist among the mechanisms you are attempting to measure in the laboratory.

2-5. **Oxygen-Carrying Capacity.** The first thing most people think of when the functions of blood are mentioned is its capacity to supply oxygen to body tissues. This is indeed a vital function, although we are sophisticated enough to know that unless all mechanisms function properly homeostasis cannot be maintained. So in this sense, we cannot very well say that carrying oxygen is the most important function of the blood. At least we can agree that carrying oxygen is a vital role for which the erythrocyte is uniquely equipped.

2-6. The exchange of gases between the alveoli of the lungs and the bloodstream is referred to as

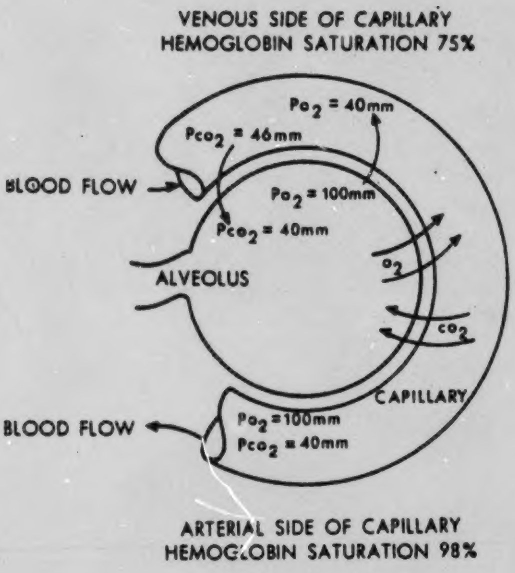

Figure 1. External respiration.

external respiration and is pictured in figure 1. The exchange of gas between the blood and tissue cells is called *internal respiration*. Because respiration is a principal function of blood, we will now examine this process more closely. Please note that we are not using the term "respiration" in the popular context of "breathing."

2-7. *External respiration*. The gases with which we are concerned in the lungs, the blood, and the cells are nitrogen, oxygen, carbon dioxide, and water vapor. It is convenient to discuss these gases in terms of their pressures or tensions. By doing so, the gas exchange mechanism—pressure gradients—can be more easily understood. Average values of the pressures of these gases have been determined by experimentation, and it is most important to have a knowledge of these pressures in the four places mentioned in table 1. This table shows the average values in the alveoli, the arterial blood, the venous blood, and the tissue cells.

2-8. When air is breathed at a normal sea level pressure of 760 mm. Hg., important changes begin to occur as the air enters the conducting passages and progresses toward the alveoli. Since the passageways contain water vapor at a pressure of 47 mm. Hg., the air is diluted by the water vapor, and consequently the total air pressure is reduced by that amount (760 − 47 = 713). The moist air, now at a pressure of 713 mm. Hg., enters the bronchial tree and then the alveoli. The alveoli, however, contain carbon dioxide at an average pressure of 40 mm. Hg., and nitrogen at its constant pressure of 573 mm. Hg. The oxygen in the inspired air just prior to entering the alveolus has a pressure of approximately 150 mm. Hg. (21 percent of 713). The carbon dioxide present further penalizes this oxygen pressure until it has a value of 110 mm. Hg. This value is called the "calculated" alveolar partial pressure of oxygen. It has been proven experimentally that this calculated value is slightly high. The alveolar partial pressure of oxygen most frequently used is *100 mm. Hg*. The slight difference is probably due to the fact that neither the atmospheric composition nor the action of carbon dioxide and oxygen in the al-

TABLE 1
AVERAGE VALUES OF GAS TENSIONS IN THE BODY

Gas	*Alveolus* *mm Hg*	*Arterial Blood* *mm Hg*	*Venous Blood* *mm Hg*	*Cell* *mm Hg*
Oxygen	100	100	40	1-60*
Nitrogen	573	573	573	573
CO_2	40	40	46	46
H_2O Vapor	47	47	47	47

Note: The value for nitrogen is constant; therefore, there is no exchange of this gas during the respiratory phases.

*Value controlled by tissue activity.

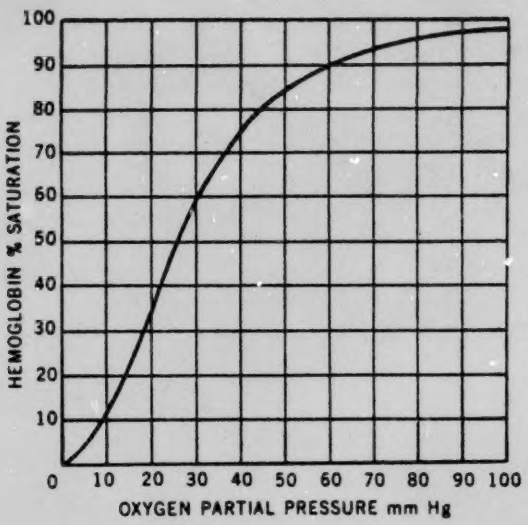

Figure 2. Dissociation curve of hemoglobin.

veoli is static, and also to other slight physiological variances.

2-9. If the inflation of the alveolus is considered to be static at a given point, figure 1 shows the relationship of the various gas pressures both within the alveolus and the bloodstream. The exchange of oxygen between the alveolus and the capillary is due to the great difference between the pressures. Since, according to the Law of Gaseous Diffusion, gases will flow from a high pressure area to one of lower pressure, oxygen diffuses through the walls of the alveolus and into the bloodstream. The pressure gradient is 60 mm. Hg. Oxygen is constantly diffusing into the capillaries around the alveolus, and the exchange continues until a value of 100 mm. Hg. is reached within each capillary. At this point the hemoglobin of the red cells is saturated to approximately 98 percent of its capacity, as shown in figure 2. It should be noted that the oxygen pressure in the venous side of the capillary is 40 mm. Hg. The percent saturation of the hemoglobin in this part of the capillary is, therefore, 75 percent (see fig. 1). This process is one of oxygenation (not oxidation).

2-10. At the same time that the exchange of oxygen is occurring, there is a similar transfer of the waste gas, carbon dioxide. The pressure of carbon dioxide in the venous blood is 46 mm. Hg; in the alveolus it is 40 mm. Hg. The exchange will proceed from the capillary blood into the alveolus. Although the carbon dioxide pressure gradient is only 6 mm. Hg., a large quantity of gas flows across into the alveolus. This is explained by the fact that carbon dioxide has a greater coefficient of diffusion than does oxygen. As this exchange continues throughout the course of the capillary, the pressure of carbon dioxide in the blood falls until it reaches its normal value of 40 mm. Hg., as noted in figure 1.

2-11. As the two gases flow in and out of the alveolus, the blood is oxygenated. The oxygen content is high and the carbon dioxide content is low. The blood then returns to the left side of the heart to be pumped throughout the body to all tissue cells. At this level, the second phase of respiration will occur—internal respiration.

2-12. *Internal respiration.* We will now turn our attention to the exchange of gas between the blood and the tissue cells. The "pure" highly oxygenated blood with a low content of carbon dioxide leaves the left side of the heart with each contraction and travels throughout the arterial system to all of the capillaries.

2-13. The cells of all types of tissues are in contact with capillaries. As it enters the arterial side of the capillary, the arterial blood has gas tensions as follows: oxygen 100 mm. Hg., carbon dioxide 40 mm. Hg. These values are noted in figure 3. In the cell, oxidation is constantly occurring and consequently, the oxygen content is at a low level. It varies in value depending upon the activity of the cell at the time. A muscle cell, for example, during exercise would have a lower oxygen pressure than during a period of rest. The oxygen tension can vary from about 1 mm. Hg. to about 66 mm. Hg. Since the oxygen pressure in the blood on the arterial side of the capillary is always higher than in the tissue cell, a large pressure gradient exists and there will again be a flow of oxy-

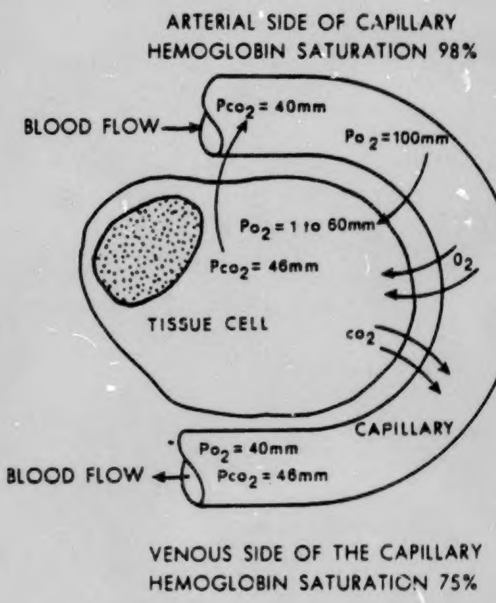

Figure 3. Internal respiration.

gen to the lower pressure—in this case, from the blood into the cell. This transfer of oxygen is actually occurring throughout the length of the capillary until the pressure of oxygen in the blood gains equilibrium with the oxygen pressure in the cell.

2-14. While the transfer of oxygen is occurring, there is also a transfer of carbon dioxide. The constant metabolic activity of the cell results in a high production of carbon dioxide. The tension of carbon dioxide reaches 46 mm. Hg. In the arterial blood in the capillary, the tension of carbon dioxide is about 40 mm. Hg. Even though a much smaller pressure gradient exists for this gas than for oxygen, a larger amount of carbon dioxide will be forced from the cell into the blood, due to the diffusion characteristics of carbon dioxide. As this transfer continues, the carbon dioxide tension in the blood increases to a level of about 46 mm. Hg. Some of the carbon dioxide combines with hemoglobin and some is carried in the plasma. Thus, with the total exchange completed as the arterial blood passes the cell and enters the venous system, the blood will return to the heart to be pumped to the lungs via the pulmonary artery for another cycle of gaseous exchange. External respiration, as illustrated in figure 1, will then be repeated.

2-15. The structure of hemoglobin was discussed briefly in CDC 90411. You will recall that hemoglobin is a complex molecule with a molecular weight of 64,500 atomic mass units (a.m.u.). Four of its 10,000 atoms are iron, and each of these can carry 2 atoms of oxygen. Heme groups and chains of amino acids surround the four atoms of iron. The protein portion of the molecule constitutes the globin and functions in the transport of carbon dioxide.

2-16. There are many types of hemoglobin, some of which are characteristic of certain stages of life. We are born with a hemoglobin known as "F" for fetal. As a person matures, this changes to hemoglobin A. This is the so-called normal form of hemoglobin. There are many abnormal forms of hemoglobin which can be identified by electrophoretic techniques. Best known of these are hemoglobin "C" which is associated with Mediterranean anemia and hemoglobin "S" identified with sickle cell anemia.

2-17. **Erythrocyte Metabolism.** As we stated earlier, the red blood cell is not just a metabolically inert hemoglobin-carrying particle. On the contrary, the erythrocyte is a biologically active cell which itself respires, does work, and carries out biochemical reactions much the same way as any body cell. Energy is required to accomplish the active transport of glucose and ions against a gradient across the red cell membrane. The red cell must also supply energy in reducing and converting iron to the ferrous state. As hemoglobin oxidizes, it shifts to the ferric state, thus converting to methemoglobin. The shift and the reverse process may be diagrammed as follows:

Reduced Hemoglobin FERROUS STATE	ENERGY	Oxyhemoglobin (Methemoglobin) FERRIC STATE
Fe++	←	Fe+++
	→	
	$+O_2$	

2-18. Most of the red blood cell consists of hemoglobin. Yet the RBC does contain a number of other substances, and a deficiency or absence of one or more of these substances can result in anemia. Many enzymes have been identified from studies of the erythrocyte. For example, the effect of Glucose-6-Phosphate Dehydrogenase deficiency was explained in CDC 90411.

2-19. **Leukocyte Functions.** Leukocytes remove invading antigens (e.g., bacteria) and to some extent transport and distribute antibodies. Monocytes and all of the granulocytes have been shown to demonstrate directional movement. Their movement is subject to *chemotaxis*, which is defined as the response of living protoplasm to a chemical stimulus. This is a means of attracting cells to substances which they must either transport or engulf. The process of engulfing and destroying bacteria, or *phagocytosis*, is a prime function of leukocytes.

2-20. *Monocytes.* These cells will engulf bacteria and larger materials, including even protozoa and red cells, and are called *macrophages*. In this regard, monocytes are perhaps the most efficient phagocytes of all the cells. Monocytes contain many of the lytic enzymes that are found in microphages (granulocytes).

2-21. *Neutrophils.* Neutrophilic leukocytes are excellent *microphages*. That is, they engulf bacteria and other microscopic particles. The particles are first surrounded by cellular pseudopodia and then incorporated into a cell vacuole. There the foreign bodies mix with substances released from the cytoplasm of leukocytes. In this way the leukocyte is not injured by whatever "combat activity" is taking place in the vacuole. Eventually the granulocytes disintegrate and in inflammatory processes are succeeded by monocytes. In addition, monocytes contain lipases which enable them to dissolve the lipoid capsules of certain bacteria. Neutrophils are fully developed (mature) cells that are incapable of mitotic division. They carry on active metabolism.

2-22. *Eosinophils.* As you already know, eosinophils are found in tissue fluid as well as in peripheral blood, especially in areas where there is

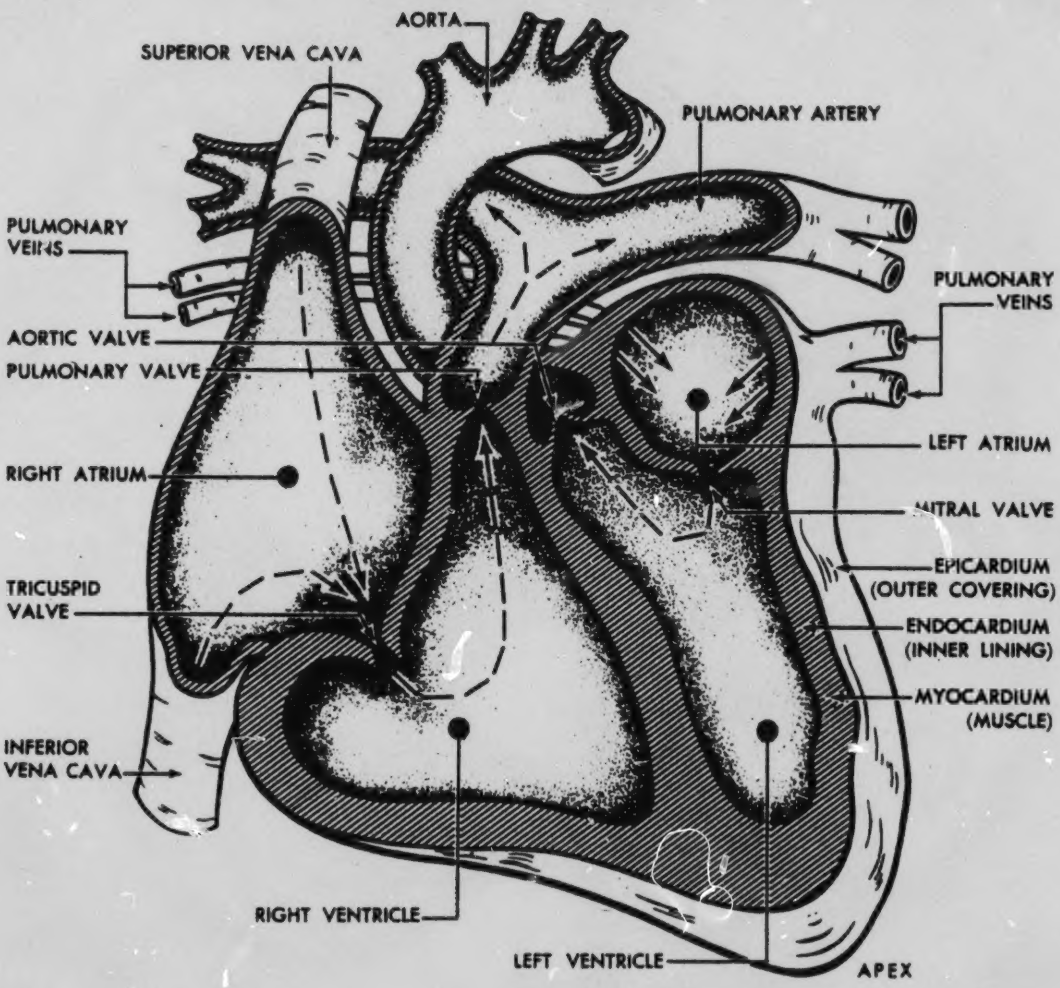
AORTA
SUPERIOR VENA CAVA
PULMONARY ARTERY
PULMONARY VEINS
PULMONARY VEINS
AORTIC VALVE
PULMONARY VALVE
LEFT ATRIUM
RIGHT ATRIUM
MITRAL VALVE
EPICARDIUM (OUTER COVERING)
TRICUSPID VALVE
ENDOCARDIUM (INNER LINING)
MYOCARDIUM (MUSCLE)
INFERIOR VENA CAVA
RIGHT VENTRICLE
LEFT VENTRICLE
APEX

Figure 4. Heart circulation.

an allergic reaction. Current thinking holds that eosinophils are involved in the antigens-antibody reaction. They have been shown to phagocytize antigen-antibody reactants. Eosinophils are also thought to transport, or at least contain, lysins which act on fibrin. It is suggested that eosinophils limit the action of substances such as histamine. How this is accomplished is not yet clear. The mobilization of eosinophils from their reserve in the bone marrow is at least in part under hormonal control. If the adrenal cortex is functioning properly, an injection of adrenocorticotropic hormone (ACTH) results in a marked decrease in the number of circulating eosinophils and in the number of circulating lymphocytes. On the other hand, there is an increase in the number of circulating neutrophils.

2-23. *Basophils.* The function of basophils in man has not been ascertained. They quite possibly represent a vestige of evolution. Their granules have been found to contain heparin, and these cells frequently appear during the clot dissolution phase of an injury. Hence, it has been suggested they may be involved in clot absorption.

2-24. *Lymphocytes.* The lymphocyte is now believed to be directly connected with antibody production. Undoubtedly, the lymphocyte performs important immunologic functions. According to very recent studies, many of the activities previously thought to take place in the reticuloendothelial (RE) system actually take place in lymphocyte tissue.

2-25. *Platelets.* Platelets possess metabolic systems, expend energy, and respond to stimuli. They contain many enzymes and undergo respiratory activity and glycolysis. They possess coagulation factors usually designated as PF–1, PF–2, and on through PF–7 (at the present time). Some of the earlier, less specific terms like thromboplastinogenase have been dropped from usage. The cells contain fibrinogen and vasoconstrictor substances, calcium, and many other components which are either known or presumed to participate in the clotting mechanism. Clot-promoting lipoproteins are also found in platelets. In addition, well-defined antigens have been found in platelets. This partially explains the occurrence of incompatability reactions when platelets are infused into the blood during a transfusion. In Chapter 5 of this volume, we will consider the role of platelets in the blood coagulation mechanism.

2-26. **Circulation.** In concluding this chapter we will review the mechanical factors of blood circulation. Blood can perform its functions only if it reaches all body tissues and follows a regular circulatory pattern which permits oxygenation and removal of waste products. The circulation of blood depends upon several factors. Most important are the rhythmic beating of the heart; the volume of blood circulated; the condition of the blood vessels; and a system of valves to control the direction of flow.

2-27. The heart causes blood to circulate in this way: When the muscles of the atria contract, blood is forced into both ventricles simultaneously. The wave of contraction then passes to the ventricles which also contract and force the blood out into the arteries. Thus, blood is pumped throughout the entire body. Blood is circulated through both *pulmonary* and *systemic* circulation. As you read the descriptions trace the course of blood in figure 4.

2-28. *Pulmonary circulation.* Pulmonary circulation, sometimes called lesser circulation, is the circulation of blood through the lungs for the purpose of oxygenation. All venous blood returning from the body enters the right atrium through the superior vena cava or the inferior vena cava and then enters the right ventricle through the atrioventricular (tricuspid) valve. As the right ventricle contracts, venous blood is forced through the pulmonary valve to the pulmonary artery which carries the blood to the lungs, where the blood, through diffusion, exchanges waste carbon dioxide for oxygen. The richly oxygenated blood is then returned to the left atrium via the pulmonary veins.

2-29. *Systemic circulation.* Systemic circulation involves the circulation of blood from the left ventricle to all parts of the body and then back to the right atrium. When the left ventricle contracts, blood is forced through the aortic valve into the aorta. From here it takes different courses to all parts of the body through arteries, arterioles, and capillaries and then returns by way of the veins to the right atrium. Systemic circulation includes *coronary* and *portal* circulation.

- Coronary circulation involves the circulation of blood through the muscular tissues of the heart.
- Portal circulation involves the passage of venous blood from the gastrointestinal tract and spleen, through the liver and out to the inferior vena cava through the hepatic veins.

2-30. *Blood pressure and blood volume.* During contraction of the atria, or atrial systole, blood is forced through the atrioventricular valves into the ventricles. The resulting pressure is termed systolic pressure. In a normal adult male this systolic pressure as measured in the bronchial artery ranges from 110 to 150 mm. Hg. Atrial systole is followed by atrial diastole in which blood flows into the empty atria. The normal range for diastolic pressure in the adult male is 70 to 100 mm. Hg. Blood pressure depends upon blood volume. As volume is diminished, the blood pressure falls unless there is a compensating decrease in the lumen of the blood vessels.

2-31. Methods used to measure blood volume have the disadvantage of being either complex or inaccurate. Various dye dilution procedures may be found in the experimental literature, but none of these methods is widely used in clinical practice. Determining the exact amount of dye injected and recovered presents technical problems. The Ashby differential aggultination method has been used in experimental work. The method is hazardous because it involves transfusing the patient. A similar technique which involves infusing Cr^{51}-labeled cells is becoming more popular. Radioactive phosphorus has also been used. As noted in current research on plasma volume and red cell mass, single tracer methods may not be entirely reliable either.[2] While we will not describe volume determination in any further detail, it is most important that you realize the significance of blood volume studies and the precision required in measuring total blood volume. It should also be apparent to you that venous blood differs somewhat from arterial blood. For example, the CO_2 differs. Recent studies have shown that blood taken from a properly performed "finger stick" does not differ significantly in most routine hematologic values (e.g., hematocrit) from venous blood.[3] This is because blood that is obtained from a cutaneous fingertip puncture comes mainly from metarterioles and venules.

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[2] "Simplified Method For Simultaneous Determination of Plasma Volume and Red Cell Mass with I-Labeled Albumin and Cr-Tagged Red Cells." Grable E. and Williams J. A., Beth Israel Hosp., Boston, Mass. *J. Nucl. Med.* 1968, 9/6 (219–221).

[3] Mostert, J. W.; Trudnowski, R. J.; Lam, F. T.; and Moore, R. H. *Anesthesiology.* 29:1, 1968.

2-32. Hemoglobin values alone can often be misleading. Suppose a patient suddenly begins to bleed profusely. The blood volume would drop, and the patient's blood pressure would also drop. Until tissue fluids move into the vascular system, or until the patient receives fluids I.V., the hemoglobin and hematocrit will not *immediately* change. This is because the results of these tests are reported in terms of *concentration* rather than total blood volume. While the concentration of cells hasn't changed significantly, the total number may be markedly changed.

2-33. This can be seen in the following example. A patient loses 2 pints of blood due to an acute injury. The 2 pints of blood contain both a certain number of cells (concentration) as well as a certain amount of plasma (volume). If we perform a hemoglobin on the patient and repeat the test 30 minutes later the test results will likely be similar. This happens because the amount of hemoglobin in a unit of plasma has not changed since both cells and plasma were lost at the same time. Later, when the body replaces the lost blood volume the concentration of hemoglobin will decrease. Variations in concentration/blood volume is sometimes due to deviation in the testing procedure and may well be within acceptable limits.

CHAPTER 2

The Complete Blood Count and Related Studies

THE MAJORITY of work in hematology consists of complete blood counts (CBCs). In view of this, the technician must master procedures inherent in a CBC and know the normal values. The complete blood count consists of five tests: the red cell count, the white cell count, hemoglobin, hematocrit, and the differential white cell count.

2. Blood can be drawn by venous or capillary puncture. The choice of specimen is often determined by the amount of blood needed to do the tests ordered by the physician, as well as by the age and condition of the patient. Generally, venous samples are preferred for laboratory analysis, since they provide enough blood to perform multiple laboratory procedures or to repeat procedures for accuracy. However, capillary specimens are quickly obtained without disturbing a vein which should be kept free of scars for a potentially greater need. If you use capillary blood, it is important to have a free-flowing puncture. Capillary specimens, taken properly, will give hematology results comparable with venous specimens.

3. Collecting a Blood Sample

3-1. We will discuss aspects of getting a good sample, preserving it, and doing the tests correctly. It is always worthwhile to reaffirm good technique, since one can easily develop undesirable habits when performing simple procedures. This is especially true when the workload is heavy.

3-2. **Capillary Puncture.** Capillary puncture is a common method of collecting a blood sample. *If correctly performed,* it is easy, simple, and causes less pain and anxiety to the patient. Blood cell distribution is the same as that normally found in venous blood. A capillary blood sample is not usually treated with anticoagulant (except in the microhematocrit), and this lessens the possibility of cell distortion.

3-3. Several different sites are suitable for capillary puncture. The lateral surface just beyond the distal joint of a finger is the best site. The center part of the finger is most sensitive, and for this reason should be avoided. Heavily calloused areas or areas with excessive tissue fluids (edema) result in poor samples. The lobe of the ear may also be used for capillary puncture. Differences in cell concentration do occur when blood is obtained from this site, primarily because of a high lymphocyte concentration in the ear lobe. And too, it is not convenient to work with a patient's ear. Because of the small size of the finger, in an infant the preferred puncture site is the heel or big toe. A modification of the normal technique that has proven quite satisfactory when working with infants is to make two incisions in a criss-cross fashion or "T" if a fairly large quantity of blood is required. The Bard-Parker blade is effective, although its use is somewhat dangerous because of the nearness of tendons found in the heel region. It is probably better to use an ordinary hemolet unless you are performing microchemistry studies or other procedures in addition to a CBC which require more blood. Adequate medical supervision and training is a prerequisite to use of the "T" incision. Remember, capillary blood must be collected from a *free-flowing* puncture wound; otherwise, the laboratory results will not be valid.

3-4. **Venipuncture.** The first step in performing a venipuncture is selection of the best vein. Select a vein that is large, readily accessible, and sufficiently close to the surface to be seen or palpated. Do not guess at the location of the vein or attempt to find it by probing. In a clean, atraumatic venipuncture the needle goes directly into the vein. There is no probing or tearing of tissue. Atraumatic technique is necessary to obtain a suitable specimen as well as for patient comfort. If you have difficulty, do not hesitate to seek help. Even the most experienced technician will have difficulty once in a while, whereas another technician may find the vein on a particular patient without trouble.

3-5. It is important that correct technique be used to avoid unnecessary pain to the patient and prevent tissue damage. It is also imperative to secure a good blood specimen and to prevent contaminating the specimen or infecting the patient. A cardinal rule in the practice of medicine for which you fulfill a supporting role is to avoid harm to the patient. Veins may become distended and easier to enter by allowing the arm to hang down for 2 or 3 minutes, by massaging the blood vessel toward the body, or by gently slapping the site of puncture. Young and vigorous persons usually have elastic veins well filled with blood. Elderly or debilitated persons may have sclerosed or fragile veins, which are hard to enter or which collapse easily.

3-6. If venipuncture is a problem due to age of the patient, scarring from repeated venipuncture, or any other unusual circumstance, the technician should consult a physician. *Under no circumstances should a technician draw blood from a jugular vein or femoral vein.* When venipuncture is used to obtain blood for quantitative analyses or coagulation studies, the blood must be obtained rapidly from an area that has good circulation. If the tourniquet is kept in place for longer than 2 minutes, changes may occur in the concentration of blood components. The blood should be aspirated into a clean, dry syringe without delay. In some cases (e.g., the Lee White clotting time), siliconized syringes or syringes rinsed in normal saline are recommended.

3-7. Occasionally, the best vein is found on the hand, leg, or foot. These areas are more sensitive, however, and the veins are not as firmly anchored as those of the arm. Rolling veins may be held firm by placing the thumb on the vein so that a 1 or 2 inch length of vein lies between the thumb and the puncture site.

3-8. To do a venipuncture, you also need a cleansing agent. The most common cleansing agent used to clean the skin area over the site of venipuncture is 70 percent isopropyl alcohol. Keep in mind that alcohol does not sterilize and, in fact, is not usually sterile itself. Neither is alcohol a very effective antiseptic, but it does remove the film of natural oils and tissue debris. A precaution should be taken not to use alcohol sponges that are too wet; that is, excess alcohol (along with debris from hands) should not be rinsed back into the container.

3-9. If it is difficult to enter the vein, or if a hematoma forms, release the tourniquet, promptly withdraw the needle, and apply pressure to the puncture site. Do not reapply the tourniquet to the same arm for at least 20 minutes. Vigorous pulling on the plunger of the syrine may collapse the vein, produce hemolysis of the blood specimen, or cause air to enter the syringe. When repeated venipunctures have to be done on one patient, select different sites for blood withdrawal.

3-10. When you have finished the venipuncture, have the patient maintain pressure on the site a full 3 to 5 minutes. Be sure the sleeve of the patient's shirt or other garment is not too tight, for this can act like a tourniquet. It is helpful for the patient to hold his arm upward as if he were reaching toward the ceiling. The common technique of merely bending the elbow after venipuncture is acceptable. However, if the elbow is bent too forcefully, the clothing may bind the vein and restrict venous flow. Further, raising the arm upward will slow blood flow and minimize leakage at the puncture site.

3-11. Blood drawn by venipuncture is often stored for a period of time before it is analyzed. For this reason, you should take the following precautions to insure a valid analysis. Before withdrawing blood from its container, mix the blood sample thoroughly but gently. Blood tubes should be tightly stoppered at all times to prevent contamination and possibly even evaporation. Store the blood specimen in a refrigerator, except that serum for cold agglutinins should be separated from the clot before low temperature storage. Blood counts should be done within 3 hours after collection, and specimens taken for a CBC should never be stored overnight.

3-12. **Anticoagulants.** The choice of anticoagulant will depend on the analysis to be made. Ethylene-diamine-tetra-acetate (EDTA) is the anticoagulant of choice for a CBC. This anticoagulant causes a minimum of distortion to the cells and platelets. It does not dissolve quickly in blood, however, so the tube must be inverted four or five times when blood is added. The dipotassium salt is prepared as a 1 percent solution in distilled water, and a final concentration of 0.5 ml. of anticoagulants for each 5 ml. blood is used. Another common anticoagulant is ammonium-potassium oxalate (Heller and Paul double oxalate). This combination of oxalates does not shrink or enlarge the red blood cells appreciably. It is essential, however, to add an optimal volume of blood, no less than 3.5 nor more than 6.0 ml. As you will recall from your study of clinical chemistry, ammonium oxalate cannot be used when tests for urea nitrogen are to be done.

3-13. Heparin does not alter the size of cellular components. It is, in fact, technically ideal as an anticoagulant and is the standard for comparison of anticoagulant distortion. Heparin is more expensive and dissolves less readily than double oxalate salts. Approximately 0.5 to 1.0 mg. is required to anticoagulate 5 ml. of blood for 72 hours. The quantity of anticoagulant noted above in each case is sufficient to prevent clotting of the blood specimen. On the other hand, an excess of anticoagulant should be avoided because too much will result in distortion of cells and hemolysis. Ideally, differential blood smears should *not* be prepared from blood that contains an anticoagulant.

3-14. If oxalate is added to vials and dried in an oven, take great care to avoid temperatures above 80° C. Oxalates are converted to carbonates by prolonged exposure to elevated temperatures. Under normal circumstances, it should not be necessary to prepare your own oxalate solutions since prepared anticoagulant vacuum tubes are available from Air Force medical supply sources.

3-15. A correctly anticoagulated blood sample is essential to the proper performance of a blood cell count. The cellular constituents must remain free in the plasma and should be as similar as is possible to those remaining in the patient's circulation. Only after proper anticoagulation of the sample should you attempt the cell count, which is discussed in the following section.

4. Cell Counts

4-1. The process of counting cells is a significant chore in any laboratory. In this section we will discuss red blood cell counts (RBC), white blood cell counts (WBC), cerebrospinal fluid cell counts (CSF), and counts of spermatozoa in semen. We will also consider automated cell counting and some of the quality control measures that will help you achieve accurate counts.

4-2. **Red Blood Cell Count.** Because of their inaccuracy, red blood cell counts are no longer performed in most laboratories, except as part of an index determination. When performing a manual red blood cell count, use two separate pipettes and fill two chambers from each pipette. The average count is used to calculate blood indices.

4-3. When a venous sample is used, mix it thoroughly just prior to pipetting to insure uniform distribution of cells. This is accomplished by tilting the sample rather than by shaking it. Vigorous mixing should be avoided, since hemolysis may result.

4-4. If capillary blood is used, you must work rapidly after the puncture; otherwise, the specimen may clot. You would not necessarily be aware of a partial clot, but this would affect the outcome of a determination. This could result in a low cell count, a low platelet count, or a clogged pipette. Take care not to introduce tissue fluids by forcing blood from the finger.

4-5. Hayem solution should not be more than 3 weeks old, and must be filtered frequently. Occasionally, blood "leaks" from the pipette into the Hayem solution during diluting and causes serious error.

4-6. Pseudoagglutination (false clumping) of the red cells occurs in cases of abnormal plasma proteins (myeloma, Hodgkin disease) or with an increase in cold agglutinins (e.g., viral pneumonia). Warming the Hayem solution to 60° C. will counteract the effect of cold agglutinins without hemolyzing the red blood cells.

4-7. In polycythemia the number of red cells may be too great to give an accurate count if a dilution at 1:200 is used. In this case, you should draw blood to the 0.3 instead of the 0.5 mark, and then dilute to the 101 mark as usual. The resulting dilution of 1:333 is used in the calculation. In anemia, the opposite condition may be the case. Blood is, therefore, drawn to the 1.0 instead of the 0.5 mark and the sample diluted to the 101 mark. The resulting dilution of 1:100 is used in the calculation, as before.

4-8. Sources of error for the RBC manual count arise from improper collection, unclean or moist pipettes, pipettes with broken tips, failure to draw blood to the 0.5 mark, and drawing blood beyond the 0.5 mark. Blood drawn beyond the mark deposits a thin film of cells on the pipette, even though the column of fluid is immediately brought back down to the mark; this practice can produce an elevated cell count.

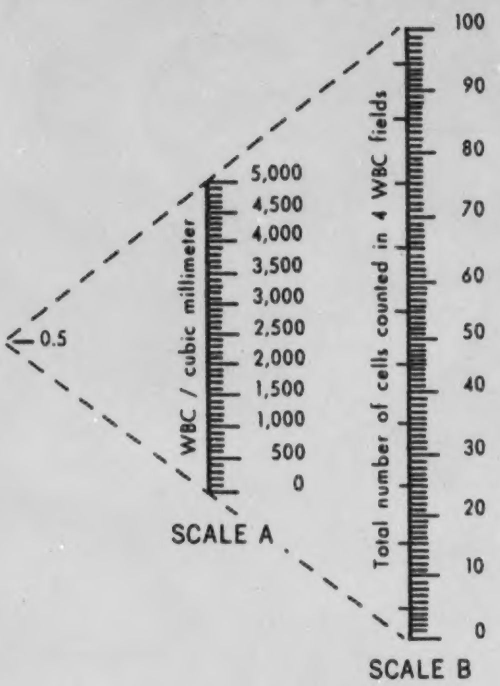
4-9. Diluting fluid should be pipetted in a continuous motion to, but never beyond, the 101 mark. The washing backward and forward of blood and diluent in the pipette to hit the 101 mark exactly overly dilutes the specimen. Slow handling of the specimen after capillary puncture allows some of the blood to coagulate. Mix the blood specimen and diluent thoroughly in the pipette just before charging a hemacytometer. Avoid overcharging or undercharging the counting chamber. Make sure you have an even distribution of cells in the counting chamber, and count all the cells carefully. Do not use diluent that contains debris.

4-10. To calculate the cell count, we use the following formula:

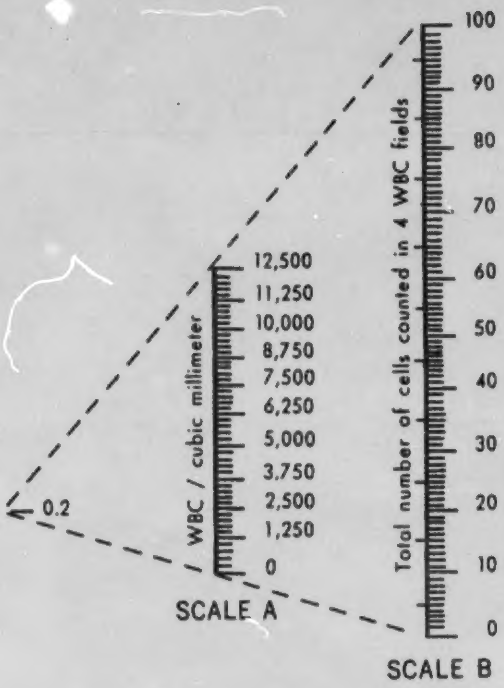
Average number of RBCs counted in the two chambers × dilution (200) × depth factor (10) × area (5) = RBCs/cmm.

The constant factors are depth, dilution, and area. Multiplying the three factors, the result is 10,000. If the standard procedure described above is followed, the number of erythrocytes counted may be multiplied by 10,000 to obtain the total count per cmm. In other words, we just add four zeros to our count. Normal RBC values in million per cmm. are as follows: birth 4.8—7.1, childhood 3.8—5.4, adult males 4.6—6.2, and adult females 4.2—5.4 (reference AFM 160-51).

4-11. **WBC Counts.** You have many occasions in your career to perform white blood cell counts manually. Be sure the pipettes are clean and dry. This is a source of error in the counting of both



(A) When blood is drawn to the 0.5 mark (1:20 dilution). Instructions: Place a straight edge from the 0.5 point in the left margin across scale A to the number of cells counted in scale B. Read the total count from scale A.



(B) When blood is drawn to the 0.2 pipette reading (1:50 dilution). Instructions: Place straight edge from the 0.2 point in the left margin across scale A to the number of cells counted in scale B. Read the total count from scale B.

I Pipette Reading	II Dilution Factor	III Multiplier	IV Normal Observed WBC Count
1.0	1:10.	25.0	200—400
0.9	1:11.	27.8	180—360
0.8	1:12.5	31.3	160—320
0.7	1:14.3	35.7	140—280
0.6	1:16.7	41.7	120—240
0.5	1:20.	50.0	100—200
0.4	1:25.	62.5	80—160
0.3	1:33.	83.3	60—120
0.2	1:50.	125.0	40—80
0.1	1:100.	250.0	20—40

(C) If blood is drawn in a WBC pipette to the figure in column I, the dilution is as shown in column II. Column III gives the figure to multiply the number of cells counted in one chamber. If the count is normal, the number of cells you would count is given in column IV.

Figure 5. Charts for circulating WBC count.

white and red blood cells. The counting chamber must be scrupulously clean and free of debris which might be mistaken for cells.

4-12. In cases where the WBC count is exceptionally high, as in leukemia, the dilution for a white blood cell count should be made in the *red* blood cell diluting pipette. Blood is drawn to the "1.0" mark, and the diluting fluid (2 percent acetic acid) is drawn to the "101" mark. The resulting dilution is 1:100.

4-13. In cases of leukopenia, the white pipette should be filled to the "1.0" mark and diluted to the "11" mark with 2 percent acetic acid. The resulting dilution is 1:10. If the number of WBCs in the chamber is still low, all 9 squares (9 sq. mm.) should be counted. Make the appropriate correction in calculation, dividing by 9 instead of 4.

4-14. The calculation for the WBC count is as follows:

$$\text{Average (of two chambers) number of WBCs counted} \times \frac{\text{dilution (20)} \times \text{depth factor (10)}}{\text{area (4)}} = \text{WBCs/cmm.}$$

Calculation aids in the form of charts and tables may be used. Some of these aids with the accompanying instructions for use are shown in figure 5.

4-15. **Cerebrospinal Fluid (CSF) Counts.** CSF is normally water clear and cell free. The specimen is collected in three test tubes, numbered in order of their collection. The third tube or final specimen collected is used for the cell count. If leukocytes are present, they may be counted and differentiated directly in a hemacytometer. Spinal fluid may also be diluted with a fluid which hemolyzes any red cells present, and examined on a Wright stained smear. With the direct method, place an undiluted drop of well-mixed spinal fluid into one counting chamber of a hemacytometer. Examine the entire ruled area for the presence of cellular elements. If both leukocytes and erythrocytes are seen, note the condition of the red cells (fresh or crenated). If the total cell content is low (less than 500), count all the cells and calculate as follows:

$$\frac{\text{Cells counted} \times \text{depth factor (10)} \times \text{dilution (1)}}{\text{area (9)}} = \text{cells/cmm.}$$

If the total cell content is high (over 500) and difficult to count, the specimen can be diluted (1/10) in a white cell dilution pipette with Hayem solution and the total cells counted. The calculation then becomes:

$$\frac{\text{Cells counted} \times \text{depth factor (10)} \times \text{dilution (10)}}{\text{area (9)}} = \text{cells/cmm.}$$

4-16. The usual technique is to draw the spinal fluid diluent to the 1.0 mark of the WBC dilution pipette; then draw a well-mixed specimen of spinal fluid to the 11 mark of the pipette. Shake the pipette for 2 minutes to mix the specimen. Overshaking may cause the white cells to clump, so this should be avoided. Charge the counting chamber and allow the cells to settle for 5 minutes. Under low power magnification, count all cells in the entire ruled area (9 square mm.). Then switch to high power magnification and examine for the differential count.

$$\frac{\text{Cells counted} \times \text{depth factor (10)} \times \text{dilution (10/9)}}{\text{area (9)}} = \text{cells/cmm.}$$

If more than 100 leukocytes per cmm. are counted, centrifuge the undiluted specimen, make a smear, and stain with Wright stain. Perform a routine differential count and estimate the ratio of erythrocytes to leukocytes. (NOTE: It may be necessary to use egg albumin or cell-free serum to make the sediment adhere to the slide.) Spinal fluid may be turbid if the cell count is higher than 500 cells per cmm. If there is gross blood with spontaneous clotting, this indicates a bloody spinal tap. Xanthochromia (yellow coloration) develops after subarachnoid hemorrhage has been present for a few hours and is due to blood pigments. Xanthrochromia may also develop from tumors, abscesses, and inflammation.

4-17. Cell counts above 10 may be significant. The lymphocyte is the predominant cell in most viral infections, syphilis, and tuberculous meningitis. Pyogenic infections due to meningococci and pneumococci usually result in a predominance of the neutrophil. Cerebral and extradural abscesses, as well as subdural hemorrhages, produce a neutrophilic response although no bacteria can be demonstrated. You should realize that to reflect the true condition of the cerebrospinal fluid, biochemical, bacteriological, viral, serological, and hematological examinations are all necessary.

4-18. **Sperm Counts.** Semen analysis also involves a cell count. However, several other aspects should be considered first in this procedure. Semen analyses may be requested by a physician in the diagnosis of infertility or as a followup test after vasectomy.

4-19. Instructions to the patient for semen collection must be explicit and explained in a professional manner. Usually the attending physician will give these instructions; however, the technician should remind the patient of several critical points. First, the patient may be required to abstain from intercourse for a period directed by the physician. The best container for collection is a clean wide-mouthed glass jar; but if a condom

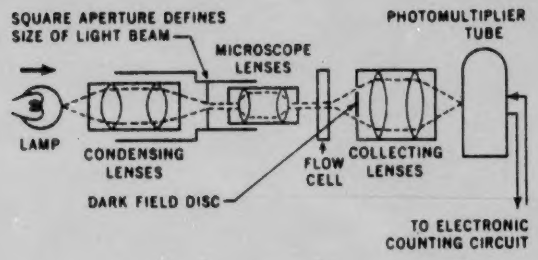

SQUARE APERTURE DEFINES SIZE OF LIGHT BEAM
PHOTOMULTIPLIER TUBE
MICROSCOPE LENSES
LAMP
CONDENSING LENSES
FLOW CELL
COLLECTING LENSES
DARK FIELD DISC
TO ELECTRONIC COUNTING CIRCUIT

Figure 6. Schematic of photoelectronic particle counting system.

must be used, it should be thoroughly washed, rinsed, and dried before using. All of the specimen must be collected, since the sperm count varies considerably in different portions. You should also explain that the specimen must be delivered to the laboratory as soon after collection as possible, preferably within 30 minutes and never over 2 hours. During this time the specimen should be kept at room temperature (25° C.) and not subjected to extremes of heat or cold.

4-20. Upon receipt of the specimen, you should record the time received and the time of collection. Then measure the volume in a 10 ml. graduated cylinder. At this time, observe and record the color (white, grey, yellow, etc.), turbidity (clear, opolescent, opaque, etc.), and viscosity (viscid, gelatinous, liquid). Finally, in this gross examination, determine the pH with a pH reagent strip and record this.

4-21. Within 15 to 30 minutes after collection the semen will liquify from the action of fibrinolysin. When the specimen has become fluid, place 1 drop on a slide and place a cover slip on it. Do not delay in performing the motility examination. Under high dry power, count motile and nonmotile spermatozoa in two or more areas. Only those which move forward actively are considered motile. Record the percent of motile forms seen. Repeat this procedure in 3 hours and 6 hours, using a new drop from the original specimen each time. Do *not* incubate at 37° C., since this impairs motility.

4-22. The sperm count is made when the specimen has become fluid. Mix the specimen thoroughly and dilute it 1:20 with a fixative solution containing 5 gm. sodium bicarbonate, 1 ml. formalin, and 100 ml. of distilled water. This dilution may be made by pipetting 0.1 ml. of semen and 1.9 ml. of the diluting fluid. Let the mixture stand until the mucus dissolves. Shake it thoroughly and load a hemacytometer. Count the spermatozoa in the same manner as you would count RBCs.
The calculations are as follows:

Sperm count × 20 (dilution) × 5 (area)
× 10 (depth factor) = sperm/cubic mm.

Then convert your answer to "sperm/ml." by multiplying the above answer by 1,000.
EXAMPLE:

155 × 20 × 5 × 10 = 155,000 sperm/cubic mm.
155,000 × 1,000 = 155,000,000 sperm/ml.

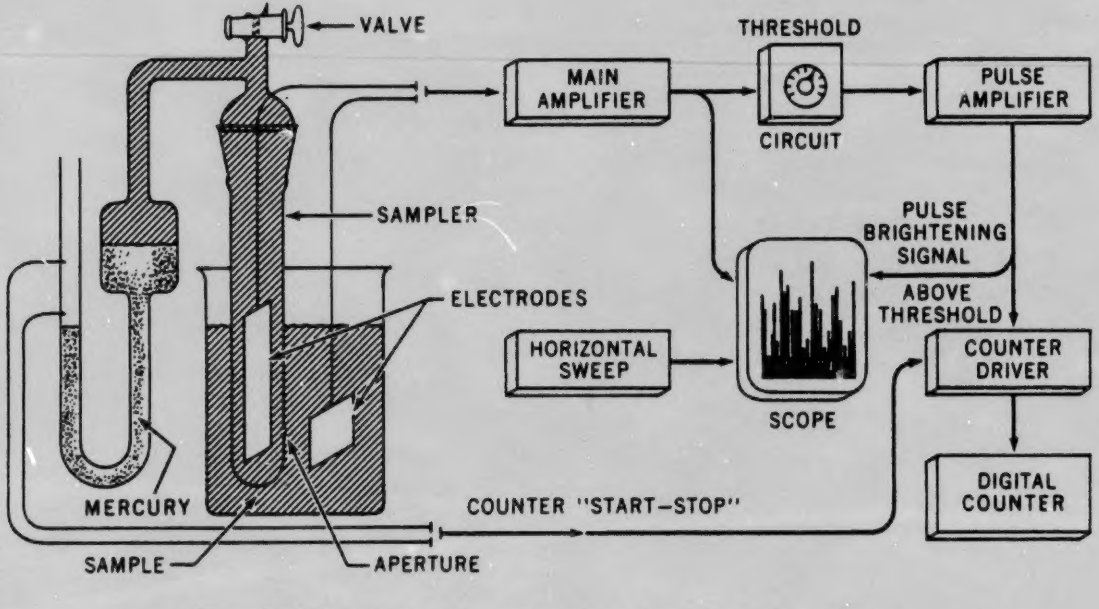

VALVE
THRESHOLD
MAIN AMPLIFIER
CIRCUIT
PULSE AMPLIFIER
SAMPLER
PULSE BRIGHTENING SIGNAL
ELECTRODES
ABOVE THRESHOLD
HORIZONTAL SWEEP
COUNTER DRIVER
SCOPE
DIGITAL COUNTER
MERCURY
COUNTER "START-STOP"
SAMPLE
APERTURE

Figure 7. Schematic of a resistance-impedance electronic counting apparatus.

Normally, a cubic millimeter of semen contains 60,000,000 to 300,000,000 spermatozoa.

4-23. **Automated Methods.** There are at least two automated methods available for counting blood cells. One of these methods is an *optical system* based upon the production of light impulses. In this system, the cells are diluted and drawn through the counting zone by a positive displacement metering pump. As cells pass through the counting area, they produce photoelectronic impulses which can be counted. Another method of automating the counting of blood cells utilizes the *principle of resistance* in an electrical field. Since blood cells are poor electrical conductors, they act as an impedence to current flow. As more cells pass into the electrical field they offer correspondingly more resistance. The change in the current flow caused by the change in resistance is sensed and counted by a digital counting apparatus. The digital counter is designed to produce numbers in a range which approximates the number of blood cells that cause the resistance.

4-24. *Devices with optical systems.* Several instruments employ a photoelectronic counting device which uses an optical sensing system and an electronic counting system. As the diluted blood specimen enters the counting area, the sample is "inspected" by a reversed dark field microscope arrangement. A schematic presentation of this arrangement appears in figure 6. When no particles are present in the sample, the narrow light beam from the lamp passes straight through to a dark field disc, which blocks further passage. However, any suspended particles, such as blood cells, will interrupt the light beam. Scattering of the light beam due to the mass of each blood cell causes a light flash to pass around the dark field disc. The light flash is focused into a detection system and converted into an electrical pulse that can be counted.

4-25. *Impedence (resistance) counters.* If a suspension of erythrocytes in an electrolyte is drawn through an aperture having electrodes on each side to form an electrical circuit, the system can be utilized to count the red blood cells in suspension. As the blood cells pass through the aperture, the mass of the cell changes the resistance between the electrodes. This changing of the resistance alters the current flow and causes electronic pulses as a result of the variation in the field. The changes are amplified, inspected, and counted electrically. The end product is a number which represents the number of blood cells in the sample being counted. By arranging the pulses and the sampling volume and sampling time, this device can be used to report directly the number of cells per cubic millimeter. Figure 7 is a schematic of the operation of one commercial instrument that counts blood cells by using the electronic principle of resistance change. When the valve is opened, the mercury falls, thus creating a vacuum in the sampler. This change in pressure causes cells in the sample to be sucked through the aperture and into the sampler. The removal of cells from the sample causes a change in electrical conductivity between the two electrodes. This change is amplified and displayed on the scope. It is further amplified and registered on the digital counter. The higher the count, the greater the probability that more than one cell will enter the aperture at one time (coincidence passage). For this reason, white blood counts over 100,000 and all red blood counts are corrected for coincidence passage.

4-26. Other methods for the automated enumeration of blood cells are currently available in instruments on the commercial market. Further refinements and developments for newer, more extensive automated techniques are undergoing research and evaluation.

4-27. *Problems of electronic counting.* One of the three most common errors of electronic cell counting lies in dilution inaccuracies. Automatic dilutors contribute an additional slight error over careful manual pipetting. However, automation does reduce the personal dilution variation among technicians. Erroneous impulses are another common problem with electronic counters. The instruments are particle counters and cannot differentiate blood cells from other solids. False impulses result from electronic problems such as inadequate electrical ground or a lack of shielding from external electronic noise. Centrifuges with worn arcing brushes are excellent electronic noise generators. A background count using a clean diluent with a known low count should identify these sources of error. Contaminating particles in apparently clear diluent are another source of impulses. A sealed bottle of saline for injection is no guarantee that the saline is suitable as a diluent. Background counts must be made on all diluents as a part of the daily quality control program.

4-28. In addition, the stromatolyzer (saponin, triton X–100, etc.) must be added to the diluent and tested to determine whether the stromatolyzer contains particulate matter as well. Membrane filters are available which will filter out particles of less than 8 microns. Diluent solutions that have a total background count (diluent + stromatolyzer) of over 100 should not be used. Cellular debris may also cause falsely elevated cell counts. An optimal concentration of stromatolyzer must be prepared. Errors are introduced also if leukocytes are affected in the process of RBC lysis. An optimal concentration of stromatolyzer is that amount which will lyse RBCs so that the RBC cellular debris is less than the WBC threshold setting for the instrument. A cell size distribution curve will determine the threshold. However, the stromatolyzer must *not* be so concentrated that WBCs are lysed before the count can be completed. Counting repetitive WBCs at 5-minute intervals will determine the time allowable before WBC lysis occurs. Usually 15 minutes is a maximum time allowance. Varous lots of saponin in the same concentration will vary considerably in their hemolyzing ability. Usually the manufacturer supplies controlled lots of hemolyzing reagent which are suitable.

4-29. Certain additional precautions should be observed when using electronic particle counters. First of all, keep in mind that running one control does not control an entire group of procedures. Errors may still be introduced with individual samples. Most laboratories allow a ±2 percent variation for different dilutions of the same sample. Bubbles must be avoided since they will be counted as cells. Count each specimen at least three times. Run a higher dilution for WBC counts over 100,000/cu. mm. and correct these counts for coincidence passage. When all is going well, the oscilloscope pattern will show steady peaks of approximately the same height. Flashes on the screen usually indicate a plugged orifice or contaminating debris. It is well to flush the orifice between counts with diluent. Replace the plastic tubing periodically to avoid cumulative contamination. Calibrating instructions for commercial instruments are available from the manufacturer. If you encounter problems such as continuous counting, failure to count, and loss of vacuum, consult the medical equipment repair personnel.

4-30. To reduce incidence of instrument failures, do the following on a daily basis:

a. Observe the mercury traveltime in the manometer. If the mercury column does not move, moves erratically, or flows quickly into the aperture tube, the manometer needs cleaning.

b. Record a background count with diluent and diluent plus saponin at the WBC threshold setting.

c. Record the control suspension counted at the RBC and WBC setting.

d. Flush orifice with dilute sodium hypochlorite *(bleach).* Flush the system thoroughly with distilled water and then saline.

4-31. In addition to the daily maintenance, other maintenance should be performed periodically. Once each week do the following:

- Oil the vacuum pump.
- Clean the orifice tube; be very careful with the orifice insert.
- Check threshold zero.

Once every 6 months you should:

- Change the latex tubing.
- Thoroughly clean the manometer.
- Check the calibration factor for the instrument.

A few crystals of thymol or filtration through a membrane will keep the saponin or other lysate free from contamination. Use only saponin that is recommended by the manufacturer of the instrument.

4-32. Using the cell count techniques described above you can rapidly determine the number of cells present in a small sample, and from this information you can calculate the number present in a larger volume. If you had to count each cell in a small drop of blood by eye, it would probably take you several days to eventually count the millions found in such a drop. Of course, as you count cells using these rapid techniques you can't tell much about them. A discussion of techniques for observing individual morphological characteristics of single cells follows in the next section.

5. Microscopic Studies

5-1. While working in hematology, you spend much of your time using the microscope. Most of your microscopic work is devoted to the routine differential. Though a clinically vital area, the differential count requires considerable knowledge and often subjective determinations. In Chapters 3 and 4 of this volume we will review the morphology of normal and abnormal cells—both erythrocytes and leukocytes. How well you understand the maturation sequence of cells will necessarily determine how well you perform differentials. However, there are even more basic considerations than identifying cells. We will discuss some of these areas in this section. You may also perform morphological studies on semen, and we are, therefore, including a discussion of this subject in this section.

5-2. **The Differential.** The routine hematology differential smear does not possess the fascination, interest, and diversity associated with bone marrow or bacteriological microscopic analysis. The smallest laboratory is required to perform a complete blood count, including routine differential evaluation of a blood smear. An intergral part of this analysis is an evaluation of atypical leukocytes, normal or abnormal erythrocytes, and the quantity and quality of platelets encountered during the leukocyte count.

5-3. Proficiency survey results in USAF laboratories and comparative studies reported in the literature indicate considerable variation among laboratories and, in fact, among technicians reporting observations from identical blood smears. This variation in differential reports is often attributed to faulty smear or staining technique. Vagaries of the Wright stain procedure may explain some of this variation, but they do not adequately account for wide differences that exist among reports. The primary reason must then be differences in interpretation by the technicians.

5-4. A medical laboratory technican may become proficient in routine blood differentials only if he has an understanding of certain basic information. Since routine differentials primarily screen normal blood cells, it is necessary to recognize *all* the characteristics of a *normal* blood cell. This includes normal biological variation. For instance, not every lymphocyte is exactly the same size, nor do all lymphocytes have exactly the same number of azurophilic granules.

5-5. A technician must understand the language of hematology. Awareness of the terms and the synonyms in hematology is essential to a comprehensive understanding. Reference material such as AFM 160–51, *Laboratory Procedures in Clinical Hematology,* and authoritative hematology texts are available in Air Force medical laboratories or libraries.

5-6. Experience is the foremost teacher in hematology. It is readily acquired in a busy hematology section where the opportunity for differential analysis occurs frequently. Experience can be diversified and interesting if proficiency slides and material from cases of confirmed diagnoses are maintained as study sets. This study material should be available to all technicians in the laboratory.

5-7. All routine blood smears should be kept until the physicians have reviewed the differential reports. A 1-week period is usually adequate. Occasionally, a review of a specific problem slide results in findings which were not originally apparent and reinforces confidence in the laboratory by the medical staff. This practice also adds to the experience and proficiency of the technician.

5-8. Certain morphological and histochemical characteristics are utilized to differentiate blood cells. A review of the significant features will promote a better understanding of blood differentials. Cellular characteristics such as relative size, shape, cytoplasmic granulation, nuclearcytoplasmic ratio, nuclear configuration, chromatin or nucleoli are very important.

5-9. Size considerations in differentiating blood cells require a defined linear standard. The micron (.001 mm.) is usually used in reference to microscopic dimensions. Ocular micrometers are available through Air Force medical supply channels and are easily calibrated, using a hematocytometer which has standardized dimensions. In routine screening of blood smears, an experienced technician relates the size of a normocytic erythrocyte (7 to 8 microns) to the size of the white cell to be differentiated, since erythrocytes are usually present throughout the microscopic field. Finally, it should be understood that personal visual discrimination is an inaccurate gauge of linear measure. Some reference measure should be employed.

5-10. The shape of blood cells often depends upon the smear and staining technique. Variations that have no clinical significance may occur from physical and chemical distortion that result from technical error. These variations can be avoided with more careful technique. Each routine smear should be scanned initially to evaluate the smear and stain quality before differential analysis.

5-11. Cytoplasmic granulation—neutrophilic, basophilic or azurophilic—is an important morphological observation. Differences in granule color in Wright stained preparations are caused by the variable dye affinity of specific granules. The intensity of colors and the relative blueness or redness of the erythrocytes may be used to evaluate the quality of the stain. The familiar basophilic (blue), eosinophilic (red), and neutrophilic (pink) granules are quite obvious in routine blood smears. The presence, absence, type, and quantity of granules are characteristic attributes used to differentiate leukocytes.

5-12. The size ratio of nucleus to cytoplasm (N:C) is a differentiating characteristic. For instance, a cell with a nuclear mass equal to the cytoplasmic mass would have an N:C ratio of 1:1. The total cell mass is usually greater in the more immature cells and decreases as the cell matures. The nuclear mass usually decreases also as the cell matures. Of course, lymphocytes are the exception to this generality.

5-13. The nuclear configurations of leukocytes help distinguish these cells. Round, oval, indented band, or segmented are terms used to describe variations in shape. These normal configurations can be distorted by physical and chemical factors mentioned previously. Some of the leukocytes are so fragile that in thick blood smears their normal configuration may be distorted by the pressure of erythrocytes forced against them. These artifacts should be recognized as such in an intelligent evaluation of blood differentials.

5-14. Problems have arisen concerning the differentiation of band neutrophils, but there is a conventional method of differentiating them. The distinction depends only upon nuclear configuration. The precursor of the band cell is the metamyelocyte (juvenile) cell, and the more mature cell is the segmented neutrophil. The nucleus of the metamyelocyte is described as kidney or bean shaped. The nucleus of the segmented neutrophil is divided into lobes that are joined by filaments. Since the band cell lies between these two in its stage of development, any cell that becomes more indented than bean shaped and does not possess filaments must be called a band cell. The only exception would be a neutrophil more mature than a metamyelocyte in which the nucleus is folded upon itself and not completely visible. According to the rule of differential counting, this folded cell is called the more mature cell—a segmented neutrophil.

5-15. In addition to nuclear shape and size, the internal nuclear morphology shows differential inclusions. The chromatin appears finely reticulated in some cells, or as a course network, or even clumped, in others. The parachromatin, a lighter staining material beside the chromatin, may be scant or abundant. The appearance of the chromatin and the quantity of parachromatin are utilized to differentiate blood cells. The presence, absence, and number of nucleoli in the nucleus are the most distinctive characteristics of immature nuclei in blood cells.

5-16. Slides must be clean and free of oil, grease, lint, or dust to prepare good blood smears. It is best to use new slides, but sometimes it is even necessary to clean new slides in 95 percent alcohol. Blood smears are usually made from a sample of blood from the needle immediately after venipuncture. Otherwise, the sample may clot or dry. A full falling drop of blood is an excessive amount for one glass slide. Approximately half of this amount is ideal. Blood smears should not routinely be made from blood containing an anticoagulant. Anticoagulant distorts the cells and alters staining characteristics. Two or occasionally more blood smears should be made initially so that additional slides may be stained without collecting another specimen.

5-17. Use a sharp lead pencil to write the name of the patient in the thick area of the smear. A properly made blood film covers at least one-half the length of the slide but not more than three-fourths of the total length.

5-18. Different stains and different techniques are used to stain blood films. Two types of stains are in general use—those which stain fixed cells, and those which will stain living cells (supra-vital stain). The panoptic (differentiating) stains generally used in hematology are Giemsa and Wright stains. When optimal staining conditions exist, Wright stain is very satisfactory and easily differentiates cells. Wright stain consists of methylene blue and eosin dissolved in methyl alcohol. Giemsa stain may be included in the staining solution. The alcohol also fixes the blood film to the slide.

5-19. Allow the blood film to air dry completely. As you probably know, you should not blow air on the slide in an effort to enhance drying, because this may distort the cells. When Wright stain is applied, it should cover the slide, be "heaped up," and must not be allowed to remain long enough to evaporate. Wright buffer should be added to the stain in a small stream without overflowing the slide. Blowing gently on the slide sometimes is recommended if the stain-buffer mixture is not evenly distributed. A green sheen appears if the stain and buffer are properly mixed. The times for staining and buffering should be adjusted with each fresh bottle of stain to give the best results. The stain should be flushed from the slide with distilled water. Wipe excess stain from the under surface of the slide and air dry the smear.

5-20. In marked leukopenia, smears can be made from the white cell layer ("buffy coat"). In this case, you must be careful to use all of the buffy coat, and not a selected fraction which may not contain representative cells. Centrifuge the blood in a Wintrobe hematocrit tube at 500 to 800 rpm. for 5 minutes. Remove the buffy coat and make the slide in the usual way.

5-21. Slides should be stained within a few hours after they are made. If a delay of more than 3 to 4 hours is necessary, slides should be fixed in 95 percent methanol for 30 minutes. This protects the slides in areas of high humidity. Excess moisture hemolyzes red cells, while white cells become distorted or disintegrate rapidly from bacterial action.

5-22. Protect blood slides from insects, because flies and cockroaches will eat the fresh blood. If this is a problem, you should follow the procedure of fixing the slides in methanol and store them in slide boxes.

5-23. Very little actual staining takes place during the fixing stage. Most staining occurs during the buffering stage. It is important to add just enough buffer; otherwise, the smear will stain too lightly. Do not blot the smear or place it in the heat of a lamp. Heat darkens the stain and may cause cell distortion.

5-24. Microscopically, properly stained red blood cells are buff pink. If the RBCs are blue, this indicates that the stain is too alkaline. With an alkaline stain, the WBCs stain dark and have only fair distinguishing characteristics. However, abnormalities of the RBCs will be masked by the heavy stain. A dark stain may be caused by smears that are too thick, overstaining, evaporation of the stain, a stain or diluent that is alkaline, and alkaline fumes.

5-25. If the red blood cells are too red, the stain is too acid. In this situation, the white blood cells (except eosinophilic granules) stain very poorly. The tendency toward acid staining is caused by incomplete drying before staining, insuf-

TABLE 2
STAINING REACTIONS UNDER VARYING CONDITIONS

Type of Blood Cell or Component	Good Stain	Acid Stain	Alkaline Stain
Erythrocytes	Buff-pink	Bright red or orange	Blue or green
All nuclei	Purple-blue	Pale blue	Dark blue
Eosinophilic granules	Granules red	Brilliant, distinct	Deep gray or blue
Neutrophilic granules	Violet-pink	Pale	Dark, prominent
Lymphocyte cytoplasm	Blue	Pale blue	Gray or lavender

ficient staining, and overdilution of the stain with buffer. Acid staining is also often caused by prolonged washing of the slide after staining, the use of stain or buffer that is acid, and by acid fumes in the laboratory.

5-26. As a technician, you should strive for a staining reaction that is neither too alkaline nor too acid in order to acquire optimum distinguishing features for all the cells. If the staining reaction is excessively alkaline, decrease the time of staining or neutralize the stock stain solution with 1 percent acetic acid or 1 percent hydrochloric acid. If the staining reaction is too acid, increase the time of staining or neutralize the stock stain solution with 1 percent potassium bicarbonate, or a weak solution of ammonia water. Check the results on trial slides after the addition of each drop of the acid or alkalizer. A poorly stained smear may be saved by destaining rapidly with 95 percent alcohol, washing quickly in water, then restaining. Most often, it is better to start anew, making fresh stain, buffer, or both. Refer to table 2 for a comparison of properly and improperly stained cells.

5-27. In some areas, distilled or demineralized water may be used in place of a buffer solution. If acid or alkaline stains result, you should change to a buffer solution prepared with distilled water. Preweighed buffer salt tablets are available commercially, which simplify the preparation of buffers. Table 2 describes the various staining reactions.

5-28. Examine a stained blood smear under low power magnification to check the general distribution of cells and stain quality. Select a thin, well-stained area under high power magnification

TABLE 3
NORMAL DIFFERENTIAL VALUES

	Percent
Neutrophilic metamyelocytes	0-1
Neutrophilic bands	3-5
Segmented neutrophils	55-65
Eosinophils	2-4
Basophils	0-1
Lymphocytes	20-35
Monocytes	2-6

and switch to the oil immersion objective for your differential count. The intracellular morphology previously mentioned for differentiation cannot be observed adequately except with the oil immersion lens. A properly prepared smear should be thin enough so that there are few overlapping red blood cells uniformly stained and relatively free of precipitated stain. Normals for a differential count are shown in table 3.

5-29. Observe the platelets in several oil immersion fields to estimate their number and report as normal, increased, or decreased. The normal is an average of 4 to 6 per oil immersion field. If they appear significantly decreased, a thrombocyte count or coagulation test may be indicated.

5-30. Many times you may find that leukocytes are not evenly distributed over the smear. Monocytes and granular leukocytes tend to clump near the edges of the smear, while lymphocytes remain in the central portion. The cells at the extreme edge of the smear should not be counted. They are likely to be distorted and are very difficult to differentiate.

5-31. In routine laboratory work, counting 100 consecutive leukocytes on a stained blood smear is usually sufficient for a clinical workup on a patient. However, if the differential leukocyte count is abnormally high, an additional 100 white cells should be evaluated. If you count 200 cells, you may find that division by 2 for a particular kind of cell results in a decimal figure. You should not report the decimal fraction. In rounding number, always report the lesser figure for cells that are relatively rare. For example, if you count 5 eosinophils in 200 cells, report 2 instead of 3. Be sure that the total count adds up to 100.

5-32. The gradual transition from the metamyelocyte to the band and then to the segmented granulocyte makes precise classification difficult. A good rule to follow is to classify the cell according to the more mature form.

5-33. **Morphological Examination of Semen.** To study the morphology of spermatozoa, make a smear of semen and allow it to air dry. Then flush with diluting fluid to dissolve mucous and wash with Wright buffer. Stain the smear with Wright or Giemsa stain. Examine it with the oil immersion lens and record the percent of abnormal forms. The morphology examination may also be observed while doing the count. Morphologically normal sperm are quite uniform in appearance. Any sperm with rounded, enlarged, small, or bilobed heads are abnormal. Abnormal tails may also be seen as enlarged, small, irregular in length, absent, or multiple.

5-34. Morphological examinations of semen smears is included in this volume that discusses hematology because it is the most logical place to put it. Both the sperm count and the stained smear are performed with materials common to the hematology sections of clinical laboratories. The next subject for discussion, hemoglobin and hematocrit studies, are also routinely performed in hematology sections of all sizes.

6. Hemoglobin and Hematocrit Studies

6-1. The oxygen carrying capacity of blood can easily be determined in the laboratory by means of either a hemoglobin or hematocrit. In most cases, it is desirable for the clinician to know both values, especially in evaluating anemias.

6-2. **Hemoglobin Determinations.** The measurement of hemoglobin in the blood is a simple means of aiding in the diagnosis of anemia or polycythemia. It can also aid the physician in other ways, such as in assessing blood loss. It can be done quickly with a small amount of blood. Current procedures employ the principles of spectrophotometry. A flow-through hemoglobinometer is pictured in figure 8. Operation of the machine is simple. Prior to actually testing a specimen, the machine must be set using a baseline saline solution. This is done by running saline through the machine and adjusting to zero with the zero control. Next, the machine is calibrated using an amarath dye solution. The machine is adjusted to 15 grams/dl. with this calibration solution. The machine is now ready to accept the actual specimen. An internal pump sucks the specimen up through the long slender sampler on the front of the machine. An aliquot of a hemolyzing solution is then pumped from the reservoir (on the left). The specimen is mixed, hemolyzed, and passed through a filtered cuvette for reading. The reading goes to an analog computer which converts it into a numerical reading that is displayed on the digital readout register. In principle, this method measures the 3 naturally occurring hemoglobin pigments (oxyhemaglobin, reduced hemoglobin, and carboxyhemoglobin). It is not a modification of the standard cyanmethemoglobin procedure.

6-3. Because of wide variations in the hemoglobin values of normal individuals, it is impossible to give a single exact normal value. Hemoglobin values are usually expressed in grams/dl of whole blood. Significant variations in normal hemoglobin values exist in different age levels and between males and females. The range of values is presented in table 4.

6-4. For current methods employing spectrophotometry, the probable overall error of hemoglobins should be less than ±0.5 grams percent. A carefully measured manual dilution should have confidence limits of no more than ±0.3 gm. percent (2 S.D.). Pipettes that are obtained through regular supply channels cannot be assumed to be

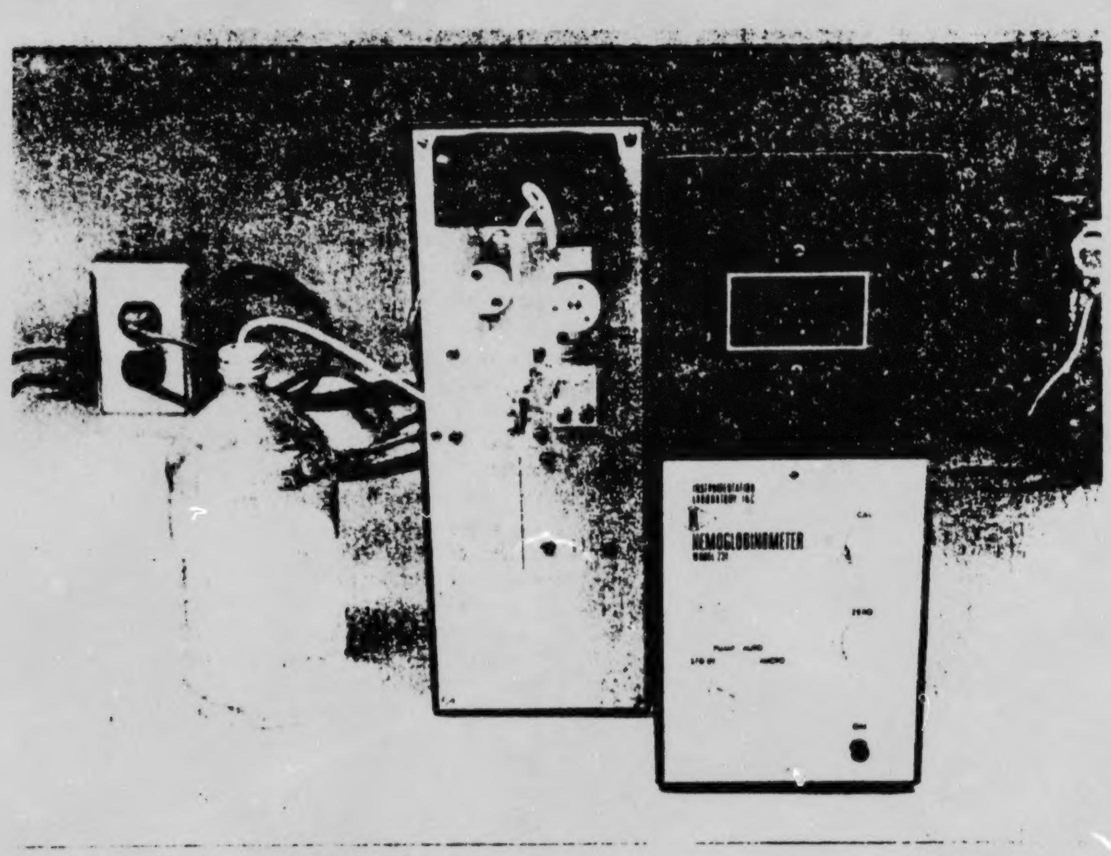


Figure 8. Flow-through hemoglobinometer.

accurate. They must be checked for accuracy. A simple way to do this is to perform a hemoglobin determination on a known sample of blood with each new pipette. If the result is out of range, repeat the procedure until you are certain about the pipette. Acceptable pipettes may be marked in some way to identify them. Technicians must not use new pipettes that have not been checked for accuracy. Experience has shown that some new pipettes will cause results to be in error by as much as 0.75 to 1.0 gram percent of hemoglobin. Pipetting errors usually affect test results significantly.

TABLE 4

NORMAL VALUES FOR HEMOGLOBIN IN DIFFERENT POPULATION GROUPS

Population group	Normal values in gm per 100 ml of whole blood
Infants at birth	18-27
Childhood	10-15
Adult males	14-17
Adult females	12-16

Human variation, as well as calibration differences, is commonly the cause of pipetting errors.

6-5. To reduce the problem of technician fatigue, variation in cleanliness of pipettes, and other recurring difficulties, various automated instruments have been developed. Repetitive dilutions may be made easily and accurately with an instrument similar to the one illustrated in figure 9. The concept of an automatic diluter involves *(a)* the sampling, or "obtaining a measured amount" from a larger volume and *(b)* the dilution of the sample. The automatic diluter is essentially two syringes that operate in a systematic manner. One syringe measures and draws the sample; the other

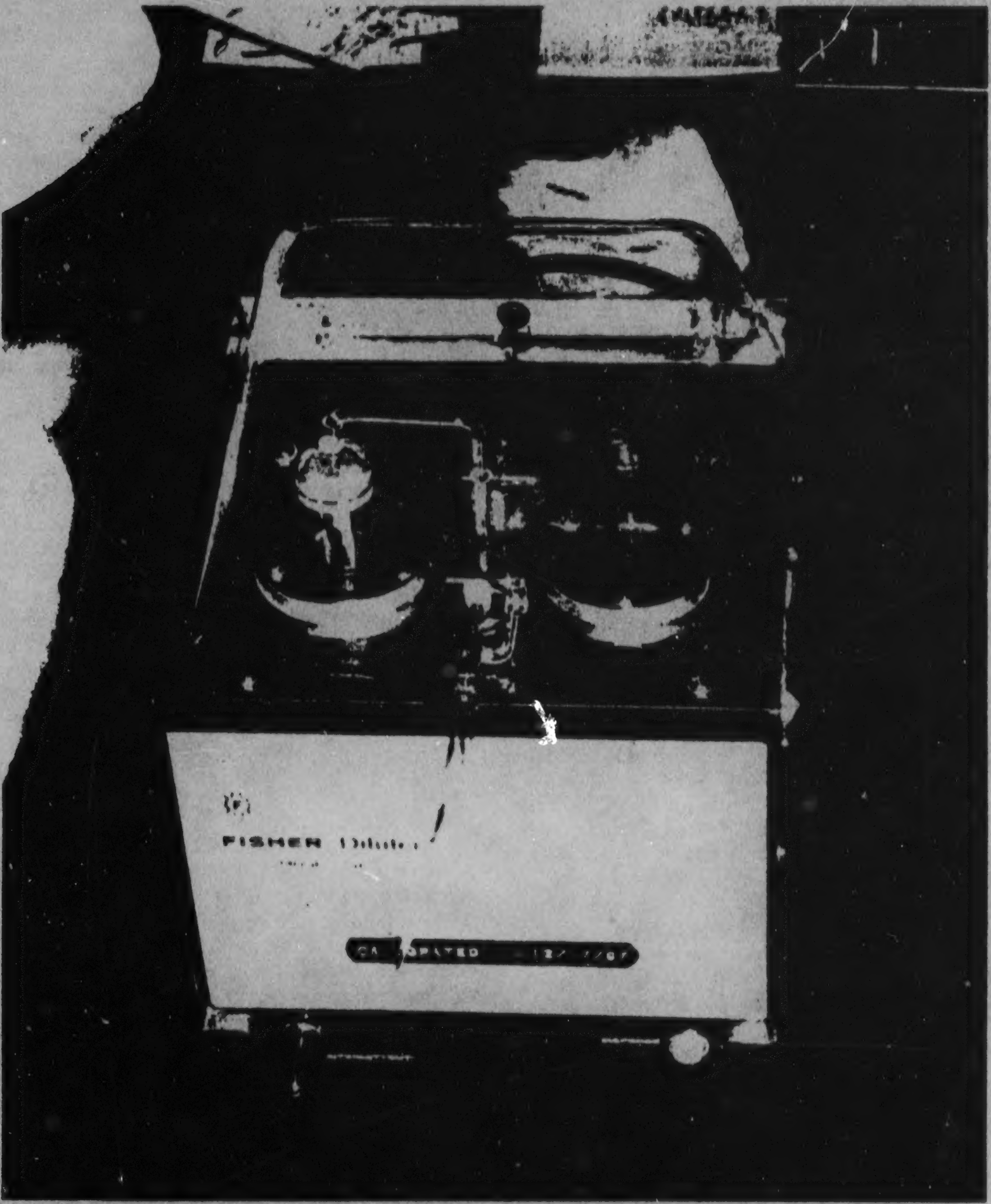


Figure 9. Automatic diluter.

syringe measures the diluent. Both of the syringes can be set to draw a metered amount repeatedly within an allowable range of error. Using the automatic diluter, the technician can "pipette" with increased reproducibility. Do not forget, however, that reproducibility (precision) and accuracy are not synonymous terms.

6-6. In the cyanmethemoglobin method, a measured quantity of blood is diluted in Drabkin solution. The hemoglobin content is then determined in a spectrophotometer.

6-7. Drabkin solution contains poisonous cyanide and must be handled with care. Cyanide salts should be stored in a secure area. Discard all solutions containing cyanide in a free flow of water to minimize contact with other chemicals, especially acids, which will release poisonous fumes.

6-8. Cyanmethemoglobin is a very stable hemoglobin pigment that does not deteriorate for several years if stored in a refrigerator. Of course, you would not expect to rely on any one standard indefinitely. The availability of commercially prepared stable standards is a distinct advantage of this method. Run daily controls as suggested in section 7; do not rely on tables or curves that are not checked daily.

6-9. Sources of error include the following: improperly calibrated pipettes, unclean cuvettes and pipettes, instrument errors (e.g., line voltage fluctuations in the spectrophotometer, loose electrical connections, faulty wavelength calibration); improper dilutions, unmatched cuvettes, and improperly prepared or deteriorated Drabkin solution. If this solution is stored in an opaque container out of direct sunlight, it remains stable at room temperature for several weeks. You should pay particular attention to the loss of color in the blank, since it is exposed continuously to light. A fresh Drabkin blank should be read spectrophotometrically against a water blank. Then subsequent readings during the day will determine whether the Drabkin blank has faded and thus resulted in falsely elevated readings of the unknown.

6-10. In a widely used oxyhemoglobin method, hemoglobin is converted to oxyhemoglobin by dilute ammonium hydroxide. The specimen is placed in a cuvette and the hemoglobin content is obtained spectrophotometrically. Oxyhemoglobin is gradually converted to alkaline hematin. This mixture cannot be calibrated without reaction time control. In addition, substances other than hemoglobin apparently affect acid hematin color. This is a secondary reaction that is accelerated with increasing alkalinity. In manual methods, this oxyhemoglobin method is less desirable than cyanmethemoglobin because the time required for complete reaction is critical. In addition, oxyhemoglobin is less stable chemically than cyanmethemoglobin. Copper ions are reported to cause this instability in both the ammonium hydroxide and sodium carbonate oxyhemoglobin procedures. Another method using $EDTA–Na_4$ reportedly eliminates this problem.

6-11. If a direct reading hemoglobin scale is not available, the standard percent transmission scale may be used. Standardize the spectrophotometer to establish a curve of percent transmission in relation to grams percent of hemoglobin. The detailed procedures for standardizing spectrophotometers and determining hemoglobin curves are supplied with each package of hemoglobin standard.

6-12. The "inactive" forms of hemoglobin (e.g., methemoglobin, carboxyhemoglobin, sulfhemoglobin) are not converted to oxyhemoglobin by any oxyhemoglobin method. This is especially pertinent in the case of heavy smokers who have an abnormally high fraction of carboxyhemoglobin. From a practical clinical standpoint, it may not be of significant value to measure carboxyhemoglobin (which is relatively unavailable for carrying oxygen in the body); however, an important consideration is the lower indices values, MCH and MCHC, which result from a lower hemoglobin value, i.e., without carboxyhemoglobin.

6-13. There are many other means of measuring hemoglobin which are not nearly as accurate as the methods previously described but still practical in some field situations. An example is the copper sulfate method in which specific gravity serves as an indication of hemoglobin content. Inaccurate methods (e.g., Talquist, Haden-Hausser, and Sahli) should not be used routinely in the clinical laboratory.

6-14. **Hematocrit.** The hematocrit is so widely used that only a few comments are necessary. This is a very important test. The relative merits of the hematocrit (Hct) versus a hemoglobin (Hgb) determination have long been argued, but as medical laboratory technicians we are concerned with accuracy and sources of error more often than which test is to be performed. If microhematocrits cannot be read promptly after centrifugation, the capillary tubes must be properly identified and placed in a vertical position or centrifuged again. Slanting of the cell layer will occur if tubes are left in a horizontal position for more than 30 minutes.

6-15. Other sources of error include inadequate sealing of the capillary tube, improper identification of capillary tubes, misreading the red cell level by mistakenly including the buffy coat, and squeezing the finger during puncture (causing dilution of specimen with tissue fluid), or failure of mix the blood if it comes from a test tube. A commercial plastic sealing material is preferred to modeling clay or heat sealing of the capillary tube. Centrifugation must be adequate to pack the cells firmly. A worn centrifuge may easily result in falsely elevated hematocrit readings, especially if the motor speed is decreased due to worn brushes or other mechanical failure. Generally speaking, it is much easier to underpack the cells than it is to overpack them.

6-16. A recently developed method for measuring the concentration of red blood cells has a wide range of application. The method is especially useful in field situations, the emergency room, aboard aircraft, and in the operating suite. It involves a battery-powered instrument that measures the hematocrit electronically. The principle of operation is based upon the insulating mass of the erythrocyte. In theory, the electrical resistance of a column of blood is a function of the relative volume percent of the red blood cells. In practice, we load a blood specimen into a sampling column and apply an electrical potential across the column. The hematocrit value is based upon the conductance of the blood plasma and the resistance of the red blood cells. In USAF laboratory operations, the electronic determination of hematocrits has proved very worthwhile. Many isotope laboratories use this electronic method exclusively when blood cell volume determinations are performed in conjunction with hematocrits. The instrumented unit has not received universal acceptance, because the microcentrifuge method is more convenient in performing a large number of routine hematocrits.

6-17. Regardless of what method is used, quality control of the procedure is essential if you are to perform an accurate test. If you observe the sources of error previously mentioned and check test results using the statistical methods discussed in the next section, a significant number of errors will be avoided.

7. Statistical Analysis

7-1. Quality control has been established as an integral part of the clinical laboratory operation. In clinical chemistry, numerous pure standards, lyophilized control specimens, and statistical evaluations are maintained and utilized in the attainment of quality production through adequate control.

7-2. Until recently, the statistical evaluation of hematology procedures was generally neglected. Of course, most hematology laboratories have insisted upon the use of stable hemoglobin standards and spectrophotometric checks of hemoglobin calibration. They also require the utilization of accurate pipettes (±1 percent error) and precise pipetting and dilution work. However, more often than not, the quality of hematology procedures has depended upon the integrity and ability of the individual technician. This may be flattering to the technician, but it is not quality control. Technicians cannot, and should not, be expected to operate with machinelike precision.

7-3. Exact reproduction of tests is not attainable, and variation in results is a demonstrated fact. The ultimate purpose of quality control in the laboratory is to understand this variation so that confidence limits may be established, erroneous results detected, and appropriate corrective action initiated to rectify any *significant* discrepancy. An adequate quality control system will indicate the degree of confidence in the test and provide ample warning when a procedure is "out of control."

7-4. As you recall from your study of quality control in chemistry, the statistician employs the term "standard deviation" to evaluate variation. In these terms, a statistician might declare that any variation greater than three standard deviations would be "out of control," or that the chances of any variation greater than this would be 0.3 in 100. The probability of any variation greater than two standard deviations (S.D.) is 5.0 in 100.

7-5. The approach to control or statistical evaluation of hematology is to employ the S.D. value as a tool. The usual mathematics employed to derive this value is tedious, but a simpler formula has been derived and, though not exact, may be used for this evaluation. The calculation utilizes the differences encountered in duplicate determinations. The formula is:

$$\text{Standard Deviation} = \sqrt{\frac{\Sigma (a - b)^2}{2N}}$$

Where:

a = first value of specimen X
b = second value of specimen X
$\Sigma (a - b)^2$ = the sum of the differences of all duplicates squared and
N = number of duplicates

The square and square root values are easily obtained from a slide rule or table which is available in most laboratories. An example of one day's run of duplicate determinations is given in table 5. Under the column labeled *Auto Hgb* are the results of five different hemoglobin determinations each of which has been run in duplicate. Using the first specimen as an example, the first step in the process is to find the difference between the duplicates:

$$\begin{aligned} & a - b \\ = {} & 15.6 - 15.4 \\ = {} & 0.2 \end{aligned}$$

TABLE 5
ONE DAY'S RUN OF DUPLICATE DETERMINATIONS

Auto HGB			Manual HGB			WBC Electronic			HCT		
	a-b	$(a-b)^2$		a-b	$(a-b)^2$		a-b	$(a-b)^2$		(a-b)	$(a-b)^2$
1 a 15.6	.2	.04	15.4	0		4550	50		46	0	
b 15.4			15.4			4550		2500	46		
2 a 16.1	.3	.09	15.6	.2	.04	6300	200	40000	48	0	
b 15.8			15.8			6500			48		
3 a 15.4	.2	.04	15.0	0		5450	250	62500	45	1	1
b 15.2			15.0			5700			44		
4 a 17.6	.3	.09	17.0	0		8650	200	40000	51	1	1
b 17.3			17.0			8450			50		
5 a 12.5	0	0	12.3	0		7700	350	122500	36	1	1
b 12.5			12.3			8050			37		
$\sum (a-b)^2$		.26			.04			267500			3
$\sum \frac{(a-b)^2}{2(5)}$		.026			.004			26750			.3
$\sqrt{\frac{\sum (a-b)^2}{2(5)}} =$											
S.D.		= ±.16			±.06			±164			±.17

This value is then squared:

$$(a-b)^2 = (0.2)^2 = 0.04$$

The same procedure is followed for each set of duplicates arriving at final squared values of (1) 0.04, (2) 0.09, (3) 0.04, (4) 0.09, and (5) none. These values are then totaled to find the sum of the differences.

$$\Sigma(a-b)^2 = 0.04 + 0.09 + 0.04 + 0.09 = 0.026$$

We then insert this value into the formula to arrive at our final answer:

$$\text{S.D.} = \pm 0.16$$

7-6. At the end of each month, the daily S.D.s are added and the total is divided by the number of daily determinations to obtain a monthly average S.D., or mean S.D. This monthly mean S.D. is subsequently averaged with the previous month's mean to approach a more accurate mean S.D. Most laboratories accept a maximum of two and sometimes three standard deviations for most tests. The mean S.D. value is accepted as one S.D. for the purpose of calculating the two S.D. value. Each of the daily S.D. values is plotted on graph paper and displayed prominently for the information and utilization of the technical and medical staff of the hospital. Standard deviations have been calculated with results shown in table 6.

7-7. The information in table 6 will assist the physician in the interpretation of hematology results. For instance, if hemoglobin determinations are performed on the same patient on consecutive days with values of 11.8 gm. percent and 11.2 gm. percent, the physician would want to know the confidence limits (or 3 S.D.) to determine the significance of this change.

11.8 − 11.2 = 0.6
Hgb 3 S.D. = 0.45 (from table 6)

The change is greater than 3 S.D., so from a technical viewpoint the change in hemoglobin concentration is significant.

7-8. For some purposes, it is convenient to express standard deviation as a percentage of the mean. This is especially applicable to the control of counts. When standard deviation is expressed as a percentage of the mean, the value is known as the coefficient of variation. $\text{C.V.} = 100 \times \frac{\text{standard deviation}}{\text{mean}}$. You undoubtedly recall the meaning of these terms from your study of clinical chemistry. Using latex particles for control of precision, many laboratories have been able to

TABLE 6
STANDARD DEVIATION VALUES FOR ROUTINE HEMATOLOGY PROCEDURES

Determination		1 S.D.	3 S.D.
Hemoglobin	Manual Dilution	± 0.1	± 0.3 gm%
	Auto Dilution	± 0.15	± 0.45 gm%
Hematocrit		± 0.56	± 1.68 cc./100 cc.
WBC Electronic		± 167	± 501 cells/cu. mm.
Platelet, direct phase		± 15,400	± 46,200 cells/cu. mm.

achieve a C.V. of about 6 to 8 percent with the *Coulter counter®*.

7-9. Quality control RBCs and WBCs are commercially available. However, duplicate counts can be used to provide a reference control for red and white cell counts. A study reported in *The Technical Bulletin of the Registry of Medical Technologists*, vol. 36, #11, November 1966, compared leukocyte counts performed by the two most common automatic instruments with counts made with the hemacytometer. Instrument variation for both normal and abnormal counts was less than half the variation experienced with a hemacytometer. One coefficient of variation represented 2.3 percent with the instruments and 6.5 percent with the hemacytometer. A daily log should be kept for all control values and a coefficient of variation computed for each cell count procedure. In this way daily changes in a laboratory's level of performance can be detected and corrected. It is important that the coefficient of variation be representative of most of the equipment, personnel, and variable laboratory conditions employed in the daily routine.

CHAPTER 3

Erythrocyte Studies

ON THE 14th of January, 1888, the patient was comatose, and had been so since the previous evening.

> Breathing was deep and irregular, and no pulse could be felt at the wrist. A drop of blood was obtained this morning with difficulty, by sticking and forcibly squeezing the finger. The fluid had a reddish amber color. . . . On microscopic examination the red corpuscles were found isolated and sparingly scattered . . . . Instead of making a more careful examination of the blood, I satisfied myself with the explanation that some serum from the subcutaneous connective tissue had been pressed out with the blood.

This quotation describes an undiagnozed case of pernicious anemia as it was reported in *The Medical News*, June 23, 1888.

2. The attending physician goes on to state that the patient died on the evening of January 17th, 1888, and adds with professional modesty, "I confess that the error of diagnosis in this case has shaken my opinion concerning the rarity of the disease in the southern section of the country. . . ."

3. Today, such an error in diagnosis would be far less likely because of clinical laboratory services and the availability of well trained technicians like yourself. In this chapter we will consider some important aspects of red blood cell studies. First, we will review the normal maturation sequence of erythrocytes and describe some of the variations in size, shape, and hemoglobin content which may be found. Second, we will examine the more basic phenomenon of erythrocyte production and destruction. Concluding this chapter is a brief resume of techniques peculiar to the study of erythrocytes, not included in Chapter 2.

4. Red cells can develop qualitative and quantitative variations. Purely qualitative variations include differences in size, shape, internal structure, and type of hemoglobin. Quantitative variations include a decrease or increase in the numbers of erythrocytes. A qualitative or quantitative change in erythrocytes may affect body metabolism by altering the oxygen-carrying capacity of the red blood cells. Anemia may result if impairment of the oxygen-carrying capacity is severe.

8. Morphology of Erythrocytes

8-1. In the production and development of red blood cells (erythropoiesis), these cells undergo a gradation of morphological changes. We study the normal complete cell maturation in bone marrow and peripheral blood to better differentiate abnormal cells including young forms in the peripheral blood.

8-2. In maturing, erythrocytes develop a biconcave disk shape which facilitates oxygen exchange of the cell by increasing the surface area of the red cell. The amount of hemoglobin in a blood sample will give us an idea of the oxygen-carrying capacity of the blood. We must correlate this test with an evaluation of the morphology of the erythrocytes. The size, shape, and a rough estimation of the hemoglobin content of red blood cells may be checked by microscopic examination of a stained blood smear. More objective studies are necessary to accurately evaluate the quantitative and measure qualitative variations which occur in normal erythrocytes. The erythrocytes contain hemoglobin, an iron-porphyrin ring compound, which readily takes up and liberates oxygen. Without the hemoglobin transport system, oxygen could not be carried in sufficient quantity by the blood to maintain body metabolism, and death would result.

8-3. **Normal Maturation Sequence.** Each of the major groups of cells in the rubricytic series is shown in foldout 1 (FOs 1 and 2 separate inclosure to this volume). Study each cell carefully as it is discussed. Terms used in Air Force laboratories to describe red blood cells are those recommended by the American Society of Clinical Pathologists and the American Medical Association. The cell descriptions are based on the appearance of well-prepared Wright stained smears of normal blood or bone marrow. The terms with some of their synonyms are as follows:

ASCP Terminology	*Synonyms*
Rubriblast	**Pronormoblast**
Prorubricyte	**Basophilic Normoblast**
Rubricyte	**Polychromatic or Polychromatophilic Normoblast**
Metarubricyte	**Orthochromic or Orthochromatophilic Normoblast**
Reticulocyte	
Erythrocyte	**Normocyte**

8-4. The rubriblast is a moderately large cell 12 to 15 microns in diameter. It has a large, more rounded, centrally located nucleus than other blast cells. The N:C ratio is about 8:1, so the amount of cytoplasm is described as scant compared to the nucleus. In the earliest forms, the cytoplasm stains light blue, but in the predominent, more mature cells, the cytoplasm stains more darkly with a pink cast, described as royal blue. The cytoplasm is granule free and limited to a thin rim around the nucleus. There is no evidence of hemoglobin formation (polychromatophilia) in the cytoplasm. The nuclear chromatin is finely reticulated in a close homogeneous mesh network with sparse, indistinct parachromatin evident. There are one to two nucleoli present; however, they may not be clearly visible. If the nucleoli are distinct they differentiate the rubriblast from the prorubricyte.

8-5. The prorubricyte is slightly smaller (10 to 15 microns) than the rubriblast and contains a round, slightly eccentric (off centered) nucleus. The chromatin is coarse and clumped with distinct parachromatin, but nucleoli are not present. The cytoplasm of the prorubricyte is deeply basophilic and stains royal blue. Cytoplasmic granules are not present and there is no polychromatophilia. The unique chromatin pattern, absence of nucleoli, and intense basophilia of the cytoplasm are the most differentiating characteristics.

8-6. The rubricyte is smaller than the prorubricyte (8 to 12 microns) with a round, eccentric, dark staining nucleus which is smaller than the nucleus of the prorubricyte. The chromatin material is course and granular with distinct parachromatin, sometimes described as a spoke-wheel pattern. No nucleoli are present. The cytoplasm of the rubricyte is polychromatophilic (blue pink or grey) due to the first appearance of hemoglobin. There are no cytoplasmic granules. The unique chromatin pattern of the nucleus and cytoplasmic polychromatophilia are the most distinguishing characteristics of the rubricyte.

8-7. The metarubricyte is smaller (7 to 10 microns in diameter) than the cells in the erythrocyte developmental series thus far discussed. The cell has a small round or sometimes bizarre (bilobed, clover leaf, etc.) nucleus which stains intensely basophilic (blue black). The chromatin is homogeneously dense with no parachromatin. The cytoplasm is orthochromatic (buff pink), with hemoglobin present in full amount. There are no granules present. The orthochromatic cytoplasm (loss of diffuse basophilia) and homogeneously intense, basophilic nucleus are the most characteristic features of the metarubricyte.

8-8. The reticulocyte is the immediate precursor of the mature erythrocyte. Normally, in adults, 0.5 to 1.5 percent of all erythrocytes are reticulocytes. When there is an increase in erythropoiesis there is an increase in these cells. This proliferation occurs following administration of iron in iron deficiency anemias and during the course of other anemias. The percentage of reticulocytes is normally higher in the newborn, ranging from 2.5 to 6.5 percent, but falls to the normal adult range by the end of 2 weeks. Reticulocytes are slightly macrocytic in comparison to other erythrocytes from the same specimen. However, the reticulum (basophilic substance from immature precursors) is not demonstrable in Wright stained preparations. Supravital stains such as new methylene blue must be used to observe reticulocytes. Supravital staining for reticulocytes distinguishes these erythrocytes from those with diffuse basophilia (polychromasia) and basophilic stippling (punctate basophilia), which are both demonstrable with Wright stain.

8-9. Whereas reticulocytes are normal in peripheral blood, diffuse basophilia and basophilic stippling are not normally found in erythrocytes except in bone marrow. As you can see from the foregoing descriptions, these terms (reticulocyte, diffuse basophilia, and basophilic stippling) are not synonymous.

8-10. Diffuse basophilia in an RBC is ordinarily caused by residual basophilic substance from precursors. As the term indicates, the basophilia (blue cast) is homogeneously distributed throughout the erythrocyte. These cells are often seen in abnormal peripheral smears when metarubricytes and more immature cells of this system are seen. This polychromasia should not be confused with a blue cast given to all RBC's in a peripheral blood smear from overstaining.

8-11. The erythrocyte on mature red blood cell (normocyte) normally averages 7 to 8 microns in diameter. The cell is biconcave, and this causes a difference in the intensity of cytoplasmic staining. There is no nucleus. The cytoplasm at the periphery of the cell is moderately buff pink (orange), while the center zone is less intensely stained.

8-12. **Variations in Erythrocytes.** The formation of red cells (erythropoiesis) is regulated by the intake of substances to build the cells, the storage of these substances, and their use. In normal erythropoiesis, the cytoplasm and the nuclei of cells grow at a synchronized rate. Individual differences in physiology and physical structure of the erythrocyte account for minor morphological changes. In certain diseases, these morphological changes may vary greatly.

8-13. Probably the most striking of these variations is that observed in megaloblastic anemias, of which *pernicious anemia* is one example. Pernicious anemia is a disease in which vitamin B_{12} is not absorbed. The erythrocytes do not mature normally and are larger than normal in people with this deficiency. The most notable characteristic of this abnormal maturation is a difference in the rates of maturation of the cytoplasm and the nucleus. The nucleus develops more slowly than the cytoplasm, so that in the more mature nucleated forms a finely reticulated chromatin net is seen in the nucleus. This is in contrast to the coarser clumped chromatin observed in more mature nucleated forms in normal maturation. Such development is termed asynchronism. The mature cell is large (about 10 microns) and is termed a *megalocyte* (macrocyte). The young cells of this series are named by adding the suffix "pernicious anemia type," i.e., metarubricyte, pernicious anemia type, etc.

8-14. Other factors may produce morphological variations in red blood cells. For example, genetically controlled variations in the hemoglobin may result in changes in morphology to sickle shapes, elliptocytes, or target cells. Iron deficiencies may cause striking changes wherein erythrocytes contain only a small thin rim of hemoglobin, e.g., hypochromia. In another variant the erythrocytes are small and spherical rather than biconcave disks. Let's discuss some of the more common morphological variations that occur in erythrocytes. A brief description accompanies each variant. These variations are readily seen when blood smears are stained with Wright stain. Refer to foldout 1 *as* you study each of the following abnormal forms of the erythrocyte.

8-15. Crenated erythrocytes have serrated or prickly outlines resulting from shrinkage of the cells. This may occur when blood films dry too slowly and the surrounding plasma becomes hypertonic. This deformity is artificial and has no pathological significance except when crenated cells are found in spinal fluid as discussed in the preceding section concerned with performing cell counts on cerebrospinal fluid.

8-16. Hypochromic erythrocytes have an increased central pallor as a result of decreased hemoglobin content. Extreme hypochromia, in which only a narrow rim of hemoglobin remains at the cell periphery, is called oligochromasia.

8-17. The term polychromatophilia (polychromasia) refers to nonnucleated erythrocytes which have a blue tinge instead of the normal buff-pink or orange color. The cytoplasm of these cells is not completely mature, resulting in abnormal persistence of the basophilic (blue) cytoplasm of the earlier nucleated stages. Polychromatophilia denotes erythrocyte immaturity.

8-18. Spherocytes are spherical in shape, have a diameter smaller than normal, have greater fragility than normal erythrocytes and are without central pallor. These cells are found in hemolytic diseases and are particularly characteristic of congenital spherocytosis, a Mendelian hereditary disorder.

8-19. The term "anisocytosis" is used to describe a situation in which we find erythrocytes of several different sizes in the same blood specimen. It is a frequent finding in many types of anemia; it is particulary pronounced in pernicious anemia where megalocytes and normocytes are found together. Cells smaller than 6 microns in diameter are spoken of as microcytes, while those larger than 9 microns are called macrocytes.

8-20. Poikilocytes are irregularly shaped erythrocytes. They may be pear shaped, elliptocytes, comma shaped, or occur in various other forms. Poikilocytosis results from abnormal erythropoiesis or from mechanical factors such as a change in oxygen tension, osmotic influences, or other forces. Crenated cells are not usually considered true poikilocytes. Hereditary anomalies may predispose the formation of poikilocytes when conditions for their transformation are optimal.

8-21. Sickle cells are abnormal red cells which assume a half-moon or sickle-shaped appearance under conditions of reduced oxygen tension. The phenomenon, due to the presence of hemoglobin "S," occurs primarily in Negroes. Sickling is demonstrated by mixing a drop of blood with a reducing agent such as a *fresh* solution of sodium metabisulfite. It is possible to demonstrate sickling in individuals who are not homozygous and hence possess the sickle cell trait but do not show clinical symptoms. Consequently, the observation of this phenomenon does not necessarily mean that the patient has the anemia. The sickle cell trait occurs in 10 percent of all Negroes in the United States, but only about 1 percent have sickle cell anemia. Hemoglobin electrophoresis, which was discussed briefly in CDC 90411, is considered a more definitive procedure. In clinical cases of sickle cell anemia, abnormal erythrocytes may be observed on the routine differential smear.

8-22. Target cells are red cells which have more deeply stained centers and borders, separated by a pale ring, giving them a targetlike appearance. Large numbers of these cells are characteristic of hemoglobinopathies but may also occur in kidney or liver disease and normally in patients who have had a splenectomy.

8-23. Howell-Jolly bodies are nuclear remnants found in the erythrocytes in various anemias. They are round, dark violet in color, and about 1 micron in diameter. Generally, only one Howell-Jolly body will be found in a red cell. However, two or more may sometimes be present.

8-24. Cabot rings (ring bodies) are blue, threadlike rings found in the red cells of patients with severe anemias. They are remnants of the nuclear membrane and appear as a ring, or "figure eight" structure. Usually only one such structure will be found in any one red cell.

8-25. Basophilic stippling (punctate basophilia) is the occurrence of round, small, deeply basophilic granules of varying size in the cytoplasm of the red cell. They are ribonuclear proteins which normally disappear as the cell matures. Basophilic stippling can be demonstrated by standard staining techniques in contrast to reticulocyte filaments which require a special stain. Stippling occurs in anemias where it denotes cell immaturity. In heavy metal poisoning (lead, zinc, silver, mercury, bismuth) basophilic stippling is apparently the result of abnormal reactions of cytoplasmic structures in the RBC precursors.

8-26. Heinz-Ehrlich bodies are small inclusions found primarily in hemolytic anemias induced by toxins. They are round, refractile bodies inside the erythrocyte and visible only in unfixed smears. It is thought they are denatured hemoglobin and indicate erythrocyte injury.

8-27. Siderocytes are erythrocytes containing iron deposits. These deposits indicate an incomplete reduction of the iron from ferric to the ferrous state that is normally found in hemoglobin. Prussian blue stain must be used to readily demonstrate these cells.

8-28. Acanthocytes are spherocytic erythrocytes with long spiny projections. This is a congenital abnormality associated with low serum concentration of low density (beta) lipoproteins. It is inherited as a recessive genetic trait.

8-29. Burr cells are triangular or crescent-shaped RBCs with one or more spiny projections on the periphery. These are seen in small numbers in uremia, carcinoma of the stomach, and peptic ulcer.

9. Abnormalities in Erythrocyte Production and Destruction

9-1. The number of red blood cells in an adult remains fairly constant. The total number or volume of erythrocytes can be measured by special tests. Knowledge of the total red blood cell volume and total plasma volume may help the physician to determine the amount of replacement blood or intravenous fluids to be given to a patient.

9-2. The red blood count based on the total erythrocyte volume may be subject to a slight, even hourly, variation. In the normal individual, this range of variation remains essentially the same throughout adult life. The rate of destruction of aged and worn out cells is approximately the same as the rate of production. If the production-destruction ratio is not in equilibrium, either more or fewer red cells than normal will result. If the number of red cells is increased, the condition is known as polycythemia; if it is decreased, lowering the hemoglobin concentration, the condition is known as anemia.

9-3. **Polycythemia.** There are three types of polycythemia. The most common is known as relative polycythemia. This term describes increase in blood cell concentration resulting from plasma loss as in burns, and dehydration from excessive vomiting, diarrhea, sweating, or stress.

9-4. The second most common form of polycythemia is known as secondary polycythemia. It is frequently a response to hypoxia (insufficient oxygen). Secondary polycythemia may result from prolonged exposure to high altitude, anoxia due to congenital heart diseases, or chronic exposure to those chemicals which inactivate the oxygen-carrying potential of hemoglobin, i.e., nitrate and carbon monoxide.

9-5. The third type of polycythemia is known as polycythemia vera, a condition of unknown cause. Polycythemia vera is characterized by a widespread stimulation of the entire bone marrow, with overproduction involving granulocytes and platelets as well as erythrocytes.

9-6. **Anemia.** Anemia can be manifested by a decrease in the quantity or an alteration in the quality of the erythrocytes. Anemia is a symptom rather than a disease; however, the classification of anemias is both difficult and complex. From your standpoint, the classification of anemias is basically concerned with size, shape, color, and inclusions of the red cells. The normal red cell is 7 to 8 microns in diameter. Variation in size of cells is called anisocytosis, as we defined it earlier in the chapter. Remember, too, that large cells are called macrocytes and the cells that are smaller than normal are called microcytes.

9-7. Normal stained erythrocytes are orange or buff pink colored due to hemoglobin content; they are called normochromic. A cell that has only a small rim of color is said to be hypochromic. Hyperchromia does not exist because the erythrocytes do not exceed more than 33 to 36 percent hemoglobin, the normal amount.

9-8. On the basis of pathogenesis, the anemias may be classified into those caused by deficient hemopoietic maturation factors (the hemoglobin deficiency anemias), and those due to deficiency in numbers of red cells. Exclusive of iron, normal hematopoiesis depends upon factors in the diet; intrinsic factor; vitamin B_{12} and folic acid; gastric absorption; and liver storage factors. Deficiencies in any or all of these factors will result in the megaloblastic maturation typical of pernicious anemia and sprue.

9-9. We have already learned that inherited abnormalities of hemoglobin (hemoglobinopathies) can cause definite anemic conditions such as sickle cell anemia and Thalassemia. Sickle cell anemia is characterized by the typical sickle-shaped cell, whereas Thalassemia exhibits target cells. Acquired hemoglobin disorders may be caused by endocrine disorders, infection, poisons, or radiation.

9-10. In hemorrhagic or hemolytic states, red cells may lose their ability to perform normal functions. Chronic, slight hemorrhage may not cause anemia, since the system frequently compensates for a small loss. However, if the loss of blood exceeds production, an iron deficiency anemia may result. This will cause the red blood cells to be smaller than normal, a condition known as microcytosis. In addition to chronic blood loss, this hypochromic, microcytic anemia may result from a poor diet, malabsorption, or poor iron utilization. With acute hemorrhage, the blood loss is so great that the body does not have ample time to compensate for the loss.

9-11. Hemolysis causes the liberation of hemoglobin and results in a shortened cellular survival. Thus, there may be an increase in the serum bilirubin, urine urobilinogen, and stercobilinogen (fecal urobilinogen). The free hemoglobin that is released affects the body's metabolism by impairment of the oxygen-carrying capacity of the red blood cells. Such an anemia is logically called hemolytic anemia. Congenital hemolytic anemia is recognized by abnormal cellular morphology (elliptocytes, spherocytes), abnormal hemoglobin ("C," "S," etc.), or both.

9-12. Acquired hemolytic anemia may be caused by bacterial or parasitic infections such as malaria; chemicals or drugs such as arsenic, lead, and sulfonamides; or from extensive burns.

9-13. Conditions causing a weakening of the cellular membrane result in hemolysis. These conditions are associated with antibodies such as the cold and warm hemolysins, and the immune substances of the ABO and Rh categories. Special tests are required for the detection of the cause of hemolysis brought on by immunologic factors. Such tests are the Coombs test, tests for cold and warm hemolysins and agglutinins, and Ham test for paroxysmal nocturnal hemoglobinuria.[1]

9-14. Rarely, the first indication of the presence of a hemolytic type of anemia is the phagocytosis of erythrocytes. Antibodies that cause hemolysis can also stimulate phagocytosis of erythrocytes.

9-15. Quite often an increased demand on the hematopoietic system due to excessive loss of blood will result in the release of immature and atypical forms of erythrocytes into the circulation. These immature forms may be an increased number of reticulocytes, basophilic stippled RBCs, or even metarubricytes. Atypical forms are those in which the rate of development of the nucleus and the cytoplasm vary from the normal.

9-16. The most common of these forms is the erythrocyte that has a homogeneous, basophilic substance diffused throughout the cytoplasm (polychromasia). Other forms result from the deposition of the nuclear material into configurations such as Cabot rings and Howell-Jolly bodies described earlier in the chapter.

9-17. Studies of the anemias are based on symptoms of the disease and the type of red cells produced. Blood indices and reticulocyte counts are helpful in evaluation of the different forms. Fragility tests that determine the degree of elasticity or strength of the cell membrance and studies of the cellular morphology are further aids to evaluation. PCV (packed cell volume) and hemoglobin studies are among the most useful tests to evaluate anemias. A few of the more common types of anemias are described below.

9-18. *Microcytic hypochromic anemia.* This is a common anemia characterized by red cells smaller than normal size and reduced hemoglobin content. This is an iron deficiency type of anemia which develops as a result of poor diet, malabsorption or poor utilization of iron, or chronic blood loss.

9-19. *Hemolytic disease of the newborn (HDN).* This is a hemolytic anemia occurring in the newborn which is usually the result of incompatibility between fetal antigens and maternal antibodies. It is characterized by hyperplasia of the bone marrow and the presence in peripheral blood of large numbers of rubricytes and reticulocytes.

9-20. *Acute hemolytic anemia.* This condition is characterized by symptoms of rapid blood destruction. It may be the result of malaria, septicemia, bartonellosis, a variety of chemical agents, extensive burns, snake venoms, or circulating antibodies. The blood picture is usually that of a normocytic anemia with anisocytosis, a high reticulocyte count, and varying numbers of other immature erythrocytes. White blood cells and platelets may also be increased. The osmotic erythrocyte fragility test is usually normal. The bone marrow is hyperplastic.

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[1] Paroxysmal nocturnal hemoglobinuria is defined as rather sudden recurring hemoglobinuria due to hemolysis during sleep.

9-21. *Congenital hemolytic anemia (hereditary spherocytosis).* This is a hereditary, hemolytic anemia characterized by an increased erythrocyte osmotic fragility, a variable number of small spherocytes, erythrocytes, and reticulocytes. Polychromatophilia and rarely metarubricytes may also be seen in the peripheral blood. Another type of congenital hemolytic anemia which is nonspherocytic results from an hereditary deficiency of glucose–6–phosphate-dehydroginase (G–6–PD) in the erythrocytes. However, it is much rarer than the congenital sperocytic anemia.

9-22. *Mediterranean anemia (Cooley anemia or thalassemia).* This is a chronic, progressive, hereditary anemia commencing early in life and affecting persons of Mediterranean origin. It is characterized by the presence of thin, target-type red cells in the peripheral blood. These cells have a decreased osmotic fragility. Immature red and white cells in the peripheral blood reflect the marrow activity.

9-23. *Pernicious anemia.* This is a chronic macrocytic, normochromic anemia caused by a defect in the production of "intrinsic factor" by the stomach mucosa. There is megaloblastic hyperplasia of the bone marrow. The peripheral blood shows macrocytosis, poikilocytosis, polychromatophilia, granulocytopenia, and granulocytic hypersegmentation. Clinical features include achlorhydria and neurological disturbances.

9-24. *Aplastic anemia.* This is a severe anemia due to interference with blood formation which may follow exposure to a great variety of chemical or physical agents (benzene-glue sniffing, arsenic, gold salts, atomic and X-radiation, and certain antibiotics), or the disease may be of unknown origin.

9-25. With few exceptions, most red cell abnormalities are observable on stained blood smears. A few, such as sickle cell anemia, abnormal sedimentation of red cells, and increased cell destruction rate, require preparations designed to measure particular characteristics of abnormal red blood cells. A few of these preparations that evaluate abnormal cells are discussed in the next section.

10. Evaluation of Red Cell Abnormalities

10-1. Several of the techniques mentioned in this chapter which evaluate or demonstrate erythrocyte morphology are used primarily as screening procedures in lieu of technically difficult, time consuming procedures. In this section we will briefly comment on a few of these methods.

10-2. **Sickle Cell Preparation.** A diagnosis of sickle cell disease must be confirmed by electrophoresis, but a simple screening test is usually used first. The screening test is based on the principle that red cells that contain hemoglobin "S" will form sickle shapes when placed under lowered oxygen tension. Persons with the sickle trait (genetically heterozygous) will show a positive sickle cell preparation even though they do not have the disease. Several other abnormal hemoglobins (i.e., Hgb-I, Hgb-C-Georgetown, Hgb-Bart) are also reported to sickle in this preparation. It is interesting to note that in homozygous "S" infants less than 4 months old, RBCs sometimes will not sickle because their fetal (F) hemoglobin has not been sufficiently replaced by adult hemoglobin.

10-3. Originally, fresh blood was sealed under a coverslip with petrolatum. After several hours, the oxygen tension lowers sufficiently, and those cells which contained the abnormal hemoglobin would sickle. Today, it is more efficient to obtain maximum sickling by mixing 1 drop of the blood specimen with 2 drops of 2 percent sodium metabisulfite ($Na_2S_2O_5$) solution. Three-grain tablets of the metabisulfite salt are commercially available and sufficient to prepare 10 ml. of reagent. It is important to prepare fresh reagent on the day it is used. The preparation should stand for 30 minutes before microscopic examination. Particular attention should be paid to the edges of the preparation before reporting as negative. The test should be reported only as positive or negative for sickling, since neither the morphology nor the number of sickled cells is significant.

10-4. **Erythrocyte Sedimentation Rate (ESR).** The sedimentation of blood has been studied since antiquity. The introduction of ESR into modern medicine came when it was proposed as a test for pregnancy in 1918. In clinical medicine today, the ESR is used in a general sense to detect, confirm, and/or follow the course of a disease process. Its interpretation involves judgments which only the attending physician is equipped to make.

10-5. The sedimentation of erythrocytes in plasma involves several interrelated factors. First, you should consider the basic physics of a solid particle settling in a solution. We know that the mass of the particle is directly proportional to its rate of fall. We also know that when the particle is more dense, the rate of fall is directly proportional to the difference in densities between the particle and the solution. In other words, an increase in mass increases the rate of fall; and the more dense a particle is in relation to the solution, the faster it will fall. In opposition to these downward forces is the viscosity of the fluid. When the viscosity is increased, the rate of fall is slower. This is an inverse proportion.

10-6. Let us relate these basic principles to the ESR. Ask yourself in what circumstances the particles (RBCs) are increased in mass. Several answers are available:

- **When roulleaux formation occurs.**
- **When autoagglutination occurs (e.g., cold agglutinins.)**
- ***In severe macrocytosis.***

Conversely, a decreased cell mass is evident with sickled cells, spherocytes, and microcytes, or in anisocytosis.

10-7. However, the relative density of the RBCs must also be considered along with the factors mentioned above. For instance, an increased mass of the aggregates (roulleaux) would increase the ESR; however, with a low MCH (mean corpuscular Hgb.) the ratio of cell density to plasma density is less, which would tend to decrease the ESR.

10-8. Viscosity further complicates our understanding of the ESR. A larger cell mass presents less cell surface to the plasma than smaller individual cells. This lowers the effect of viscosity and increases the ESR.

10-9. Because of the complexity of factors involved in the ESR, it has become evident that correction of the ESR for only anemia (reduction in total cell volume) is of questionable validity. Therefore, it is recommended by most authorities that the ESR no longer be corrected. However, since anemia does affect the ESR, though not a linear function, it is recommended that the hematocrit be reported with each ESR.

10-10. There are also several purely technical aspects of performing the ESR correctly. For instance, the ESR will vary with the length and bore size of the ESR tube. This is not a consideration in most USAF laboratories since the Wintrobe tube is almost universally used. However, if microtubes are used for infants, the normal values show a corresponding change.[2] The sedimentation tube must also be perpendicular during the test. Usually this is controlled by a leveling bubble on the tube support rack. The level should be checked before each ESR is begun. The ESR will be increased if the tube is not perpendicular. The tube rack should also be placed on a sturdy table that is not subject to vibrations from centrifuges or other equipment. Such vibrations will increase the ESR.

10-11. There are differences in ESR related to the anticoagulant. First of all, a liquid anticoagulant should *not* be used because of the dilution effect. Further, during blood collection, an optimal amount of blood must be mixed with the anticoagulant to prevent distortion of the RBCs. For instance, in a 5-ml. vacuum tube with double oxalate, no less than 3 ml. of blood must be collected. Double oxalate is the most time honored anticoagulant used; however, technically it is probably second best to either heparin or EDTA as an anticoagulant. No appreciable RBC shrinkage is reported with either heparin or EDTA anticoagulants, but up to 5 percent lower hematocrit values have been reported using double oxalate. Regardless of the anticoagulant used, it is most important to use the same one consistently with all ESRs performed in your laboratory.

10-12. Marked changes will occur in the ESR 3 hours after collection. Because of this, it is recommended that the blood specimen be used within 2 hours. The specimen should not be refrigerated or warmed. During settling of the cells, appreciable changes in the sedimentation rate will occur in temperatures outside the range of 22 to 27° C. Temperature correction charts are available to correct for slight changes in temperature alone. You should remember, however, that extremes of temperature may change certain factors, e.g., plasma protein, which also affect the ESR.

10-13. Plasma protein exerts the most clinically significant effect upon erythrocyte sedimentation. Of the plasma proteins, fibrinogen exerts by far the greatest effect. Fibrinogen causes the red cells to aggregate and thereby leads to more rapid settling. However, it is known that the influence of the plasma proteins on ESR is more a function of their relationship to each other than to their absolute concentrations.

10-14. **Red Cell Indices.** Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) comprise the erythrocyte indices. MCV is the mean volume of an erythrocyte in the RBC population of the specimen and is expressed in cubic microns. Lower values are obtained in microcytosis and higher than normal values are calculated in macrocytosis. Because MCV is a mean value, it is possible to have a normal MCV and yet have microcytes and macrocytes present (anisocytosis). The MCH is the average weight of hemoglobin in an erythrocyte of the specimen. It is expressed in micromicrograms. This value, also, may be misleading—as in the case of hypochromic macrocytosis, where the larger cells hold more than the average amount of hemoglobin but the cell is not normochromic as the MCH would suggest. In both of these calculations, it is obvious that microscopic observation must be made to validate the MCV and MCH values. MCHC is the mean percent concentration of hemoglobin in *each* erythrocyte of the population. Since a normal RBC contains the maximum concentration of hemoglobin, this value can only be reported as normal or below normal.

10-15. To calculate RBC indices, you must first have valid information to substitute in the formulas. This requires that you run duplicate determinations of the red blood cell count (RBC), hematocrit (Hct), and hemoglobin (Hgb) content. The results of these tests must agree within the confidence limits established by your laboratory. An average of the duplicate values is used in the calculations. The formulae are:

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[2] Wintrobe, M. M., *Clinical Hematology*, p. 354, 6th ed. *op. cit.*

$$\text{MCV} = \frac{\text{Hct} \times 10}{\text{RBC millions/mm.}^3}$$

$$\text{MCH} = \frac{\text{Hgb gm.\%} \times 10}{\text{RBC millions/mm.}^3}$$

$$\text{MCHC} = \frac{\text{Hgb gm.\%} \times 100}{\text{Hct}}$$

10-16. Circular slide rule calculators are available commercially which greatly simplify these calculations. Normal values for adults are: MCV, from 82 to 92 cubic microns (c.μ); MCH, from 27 to 31 micromicrograms ($\mu\mu$g.); MCHC, from 32 to 36 percent. Normal values for MCV and MCH in newborns and infants are higher. MCHC normals are the same for infants and adults.

10-17. Some difficulty has been experienced in using electronic particle counters for the erythrocyte count in calculating MCV and MCH. A particle counter should *not* be used for this purpose with the threshold set for a normocytic RBC population. Obviously, if a count is done at this threshold setting, microcytes will *not* be counted and the red cell count is invalid. More sophisticated particle counters are available which plot cell frequency against cell size, giving a cell-size distribution curve which is more meaningful. Even more complex and considerably more expensive automatic counter models are on the market, which give all the parameters of blood cell indices mentioned above in a printout record. Needless to say, the cost and maintenance of such instruments are limiting factors in their use.

10-18. **Erythrocyte Osmotic Fragility.** Red blood cells are excellent indicators of osmotic pressure. The degree of distortion or eventual lysis which occurs to an RBC in a fluid is directly related to the osmotic pressure between the cell and surrounding fluid.

10-19. An erythrocyte goes through several stages in the process of lysis. In the first stage, the normal biconcave disc crenates; then the cell becomes spheroid along with crenation. Finally, the crenated spherocyte loses its crenation and hemolyzes, and a ghost cell (representing stroma without hemoglobin) remains. Since a spherocyte has progressed to a late stage of this system, very slight osmotic changes will cause it to hemolyze. You will recall that in congenital spherocytic anemia, the erythrocyte osmotic fragility is increased. It is also increased in hemolytic disease of the newborn, chemical poisoning, and burn cases. In sickle cell anemia and several other conditions, the RBC fragility test is decreased.

10-20. The test itself is quite simple to perform. You are familiar with the term "isotonic" saline or 0.85 percent sodium chloride solution. In isotonic saline, the osmotic pressures are balanced between the red blood cell and saline so that there is no cellular distortion or hemolysis; whereas, in distilled water the osmotic pressures are so imbalanced between the two that hemolysis of the cells occurs immediately upon exposure. The osmotic fragility test is based upon these phenomena. The test consists of a series of progressively lower salt concentrations from 0.85 percent to a 0 percent solution (distilled water). When erythrocytes are placed in these solutions, the RBCs will lyse at a certain concentration below 0.85 percent (initial hemolysis), and will lyse completely (complete hemolysis) between the initial hemolysis concentration and distilled water. The salt concentrations must be very exact for clear-cut reactions. The test report should state the concentration where both initial and complete hemolysis occurred. Normally, initial hemolysis occurs between the saline concentrations of 0.42 to 0.46 percent and is complete between 0.30 to 0.34 percent concentrations.

10-21. In one modification of the fragility test, the amount of hemolysis is determined by measuring the amount of hemoglobin released. The percentage of hemoglobin in each tube is reported, equating the "complete hemolysis" tube to 100 percent released hemoglobin.

10-22. Another modification which greatly increases the sensitivity of the RBC fragility test involves incubating the blood specimen at 37° C. for 24 hours before running the test. For this modification, the blood must be collected aseptically in a sterile container with glass beads. The specimen is rotated before testing so that the blood is defibrinated by the glass beads.

CHAPTER 4

Leukocyte and Thrombocyte Maturation

EACH OF THE five types of white blood cells originate from primitive cells which have similar morphological characteristics. As primitive cells change to "blasts" and more mature types, their nuclear and cytoplasmic characteristics change. These changes are distinctive enough to differentiate the types of cells from each other. Thrombocytes (platelets) also originate from an early-type cell that undergoes changes and finally fragments into the numerous platelets found in blood.

2. A study of leukocyte and thrombocyte maturation necessarily involves studying the various stages that the cells pass through prior to assuming the characteristics of mature cells. These stages of development are generally referred to as the normal maturation sequence.

11. Normal Maturation Sequence

11-1. Developing blood cells follow a set series of events as the cell approaches maturity. Mature cells are, as a rule, smaller than immature cells. The nuclear chromatin becomes more clumped and compact as the cell matures. Also, the staining reaction of the cytoplasm changes as a cell matures. The younger cells are generally more basophilic than mature cells. Cells in the granulocytic, agranulocytic, and megakaryocytic series all demonstrate the above characteristics. These and other special differences form the basis for our discussion of the maturation of the three classes of cells mentioned above.

11-2. **Granulocytic Series.** Specific cytoplasmic granules develop in cells of the granulocytic series as the cells mature. Nonspecific granules (azurophilic) will develop initially after the blast stage and be found in early stages of the granulocytic series. As you read the description of cells in the granulocytic series, refer to foldout 2.

11-3. At birth, the granulocytic leukocytes originate in the bone marrow. A secondary potential is maintained throughout life in the reticuloendothelial system, which includes the spleen and liver. As the granulocyte matures, azurophilic, nonspecific cytoplasmic granules give way to more specific granules. We identify the stage of maturity according to the staining reaction of these granules. Normally, only band and segmented granulocytes are observed in peripheral blood. The other cells are normally seen only in bone marrow preparations. The stages in the normal maturation of the granulocytes are:

a. Myeloblast.

b. Promyelocyte.

c. Myelocyte (neutrophilic, eosinophilic, and basophilic).

d. Metamyelocyte (neutrophilic, eosinophilic, and basophilic).

e. Band cell (neutrophilic, eosinophilic, and basophilic).

f. Segmented cell (neutrophilic, eosinophilic, and basophilic).

11-4. *Myeloblast.* The myeloblast cells are about 10 to 20 microns in diameter. The nucleus is round or oval, and stains light red to purple with Wright stain. The delicate interlaced chromatin strands of the nucleus are homogeneously diffused, are without clumps, and stain evenly. Two to five distinct nucleoli are usually seen. There is a small amount of nongranular, deeply basophilic (blue) cytoplasm which forms a thin rim around the nucleus.

11-5. *Promyelocyte.* The promyelocyte cells are generally larger than myeloblasts (14 to 20 microns). The nuclear-cytoplasmic ratio is 5:1 as opposed to 7:1 for the myeloblast. The nucleoli are not as distinct as in the myeloblasts and the chromatin is slightly clumped, appearing more coarse and less evenly stained. A few large, nonspecific azurophilic granules are present in the cytoplasm. More mature promyelocytes may have a few specific granules (neutriphilic, basophilic, or eosinophilic). A cell ceases to be a promyelocyte and becomes a myelocyte when specific, definitive granules are present in the cytoplasm and the nucleus becomes slightly indented

11-6. *Myelocyte.* Myelocytes are the next more mature cells of the granulocytic series after the promyelocyte. In the myelocyte stage, there are definitive granules which may be so numerous that they hide nuclear detail. The nucleus is round or oval, and nucleoli are either not visible or absent. Myelocytes are easily distinguished as being neutrophilic, eosinophilic, or basophilic by the specific granulation.

11-7. *Neutrophilic myelocyte.* The first sign of neutrophilic myelocyte differentiation is a small, relatively light area of ill-defined, pink granules which develop in the cytoplasm among the nonspecific, azurophilic granules. As the myelocyte ages, the azurophilic granules become less prominent and disappear. The chromatin appears more clumped in the myelocyte than in the promyelocyte. Neutrophilic myelocytes are usually smaller than promyelocytes and have relatively larger amounts of cytoplasm. The N:C ratio is approximately 2:1.

11-8. *Neutrophilic metamyelocyte (juvenile).* Neutrophilic metamyelocytes have an indented or kidney shaped nucleus with many small, pink blue granules. Neutrophilic metamyelocytes are slightly smaller than myelocytes and have a relatively smaller nucleus and less defined chromatin net. Neutrophilic metamyelocytes are not seen in the peripheral blood of normal individuals, but are often found in acute conditions in which there is a marked increase in myelocytic proliferation.

11-9. *Neutrophilic band (stab).* As the neutrophilic metamyelocytes mature, the nuclear indentation becomes more marked. In the band form, the nuclear indentation is more indented than the kidney-shaped nucleus of the metamyelocyte but does not have filaments typical of the segmented neutriphil. You should recall at this point that there are normally 3 to 5 percent band forms in peripheral blood. Neutrophilic bands are slightly smaller than metamyelocytes. The specific cytoplasmic granules of band neutrophils are small and evenly distributed, and stain various shades from lilac to pink with Wright stain.

11-10. *Neutrophilic segmented cell.* Mature neutrophils are approximately twice the size of erythrocytes. These cells differ from neutrophilic bands in that the nucleus has two or more definite lobes separated by very thin filaments rather than an indentation. The cytoplasm in an ideal Wright stained preparation is buff or pink, and the small, numerous, and evenly distributed neutrophilic granules have a lilac color.

11-11. The transition between the various stages of neutrophilic cells is gradual. Differentiation of cells is made almost exclusively from the nuclear configuration. Borderline cells are difficult to distinguish. Cells should be placed in a more mature category when there is doubt; however, in the distinction between bands and segmented neutrophiles, the assumption that a filament must be present even if one cannot be seen should *not* be made. Such cells would be classified as bands. If a question arises as to whether a cell is a metamyelocyte or a band, it should be counted as a band cell.

11-12. Occasionally, the lobes of segmented neutrophils may touch one another or be superimposed. This situation may obscure the connecting filament. This is particularly true of thick films and thick areas of good blood smears. When this occurs, another smear should be used, rather than hazard "guesses" from the improperly prepared blood smear. Some consideration should be given to the use of "pulled" smears when problems arise from physical distortion of cell morphology. As a rule, well prepared "pulled" smears provide a larger area of evenly distributed blood cells than "pushed" smears. In addition, cellular morphology is less distorted over a larger area. Because of the consistency of bone marrow, it is not possible to prepare adequate marrow smears except by the pulled smear technique. There is no doubt, however, that the pulled smear technique is more difficult to master.

11-13. *Eosinophils.* These cells are characterized by relatively large, spherical, cytoplasmic granules which have an affinity for the eosin stain. The earliest eosinophils have a few dark, spherical granules with red tints which develop among the undifferentiated granules. As the eosinophils pass through various developmental stages, the granules become less purple red and more red orange. The azurophilic nonspecific granules, characteristic of the promyelocyte and the early myelocyte stages, disappear in the myelocytic stage. Eosinophils are normally found in small numbers in bone marrow and peripheral blood smears. Therefore, no useful clinical purpose is served by routinely separating the eosinophils into their various myelocyte, metamyelocyte, band, and segmented categories. In cases of eosinophilia resulting from allergies, parasitism, or leukemia, differentiation of the various stages is important.

11-14. Eosinophils seen in normal peripheral blood smears are about the size of neutrophils, and usually have a band or bilobed nucleus. The granules are spherical and uniform in size, and are evenly distributed throughout the cytoplasm, but rarely overlie the nucleus. In good Wright stain smears, the granules take a bright red-orange stain. Eosinophils can be identified without the use of stains because the granules are so uniformly round, distinct, and relatively large. It may be difficult to distinguish eosinophils from neutrophils when the granules of the neutrophils are prominent and the stain is excessively basophilic. Guess work can be avoided by the preparation and use of only Wright stain reagent which stains properly. If some attention is paid to the size, uniformity, and shape of the granules rather than to the color alone, these cells can be more easily differentiated.

11-15. *Basophil.* Basophilic segmented cells have round, indented, bandlike, or lobulated nuclei but should not usually be classified according to the shape of their nuclei. Basophils are so few in peripheral blood and bone marrow that there is no clinical advantage to differentiation into separate developmental categories. The granules of a mature basophil range in color from dark blue to black (deeply basophilic). These granules are large and irregularly shaped, completely filling the cytoplasm. Spaces may occur in the cytoplasm where the basophilic granules have dissolved in the process of staining and washing (they are water soluble).

11-16. **Megakaryocyte Series.** Microscopic evaluation of the megakaryocytes in bone marrow may be of importance in coagulation problems involving thrombocytes (platelets), since thrombocytes are produced by the megakaryocyte.

11-17. The cells of the megakaryocytic series grow larger as they mature until there is cytoplasmic fragmentation to form the thrombocytes seen in peripheral blood. Azurophilic granulation begins to appear in the second stage of development and continues until it almost obscures the nuclear lobes. The nucleus develops from a discrete round or oval shape to multiple ill-defined lobes. The stages in the normal maturation of the megakaryocytic series are:

- Megakaryoblast.
- Promegakaryocyte.
- Megakaryocyte.

11-18. *Megakaryoblast.* These cells range from 20 to 30 microns in diameter and are irregular in shape. There are two to six small nucleoli in the nucleus. The cytoplasm is scant, irregularly basophilic and agranular.

11-19. *Promegakaryocyte.* These cells are generally larger than the preceding cells, ranging up to 50 microns in diameter. They may have an indented or double nucleus. A few azurophilic granules may be present in the cytoplasm around the nucleus. The cytoplasm may show some polychromasia and initial platelet formation.

11-20. *Megacaryocytes.* These are the largest blood cells, ranging from 40 to 100 microns in diameter. They have ill-defined multilobed nuclei with clumped chromatin. The cytoplasm has irregularly dipersed, fine azurophilic granules and may show some fragmentation of platelets. Megakaryocytes do not appear in the peripheral blood. In bone marrow preparations, there should be little difficulty in recognizing cells as being of this series because of their enormous size.

11-21. **Agranulocytic Series.** The agranulocytic series is composed of leukocytes without specific granulation. This series includes lymphocytes and monocytes. Lymphoblasts, the lymphocyte precursor, originate primarily in the lymphatic system. Few are found in normal bone marrow, even though the marrow is involved in lymphopoiesis. Monoblasts are probably derived from hemocytoblasts, the precursor of myeloblasts, and from hemohistioblasts which are the most primitive cells oriented to hemopoiesis. The monoblasts mature to form promonocytes and then monocytes. Refer to foldout 2 as you study the cell types described.

11-22. The stages in the development of the lymphocytic series are:

- Lymphoblast.
- Prolymphocyte.
- Lymphocyte.

11-23. *Lymphoblast.* These cells are similar to other blast cells. The nucleus is round or oval, staining light red purple with Wright stain. The nuclear chromatin is finer than the lymphocyte but not as delicate as in the myeloblast. There is a moderate amount of light blue parachromatin. One or two nucleoli are present. The cytoplasm is moderately basophilic and nongranular, forming a thin rim around the nucleus.

11-24. *Prolymphocyte.* Some authors do not differentiate this cell from the lymphoblast. When these cells are differentiated, it is more by comparison with lymphoblasts and lymphocytes than by any unique, morphological characteristics. The nuclear chromatin is described as more coarse than in the lymphoblast, being slightly clumped. One nucleolus is usually present. There is more abundant, light blue to moderately basophilic cytoplasm. In addition, there may be a few azurophilic granules. Generally, the prolymphocyte is smaller than the lymphoblast.

11-25. *Lymphocyte.* The lymphocyte is more distinctive morphologically than its precursor. The cell is described as approximately 6 to 20 microns in diameter, or from about the size of a normal erythrocyte to more than twice the size of a red cell. The cytoplasm may vary in quantity from scant to a moderate amount, depending upon the thickness of the smear and the size of the cell. The cytoplasm is normally clear and homogeneous, and may be described as light blue, sky blue, lightly basophilic, or moderately basophilic. The cytoplasm may normally contain a few reddish-violet or azurophilic granules which are peroxidase negative. A clear perinuclear zone is often observed in

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the cytoplasm. The nucleus of a normal lymphocyte may be round, oval, or slightly indented. It contains clumped chromatin which appears in Wright stained preparations as very dark, staining bluish-purple aggregates in the nucleus separated by lighter staining, indistinct areas of parachromatin. This description of the normal lymphocyte indicates the wide biologic variations which must be thoroughly appreciated before attempting to differentiate the normal from the atypical lymphocyte. Later in this chapter, we will discuss atypical lymphocytes.

11-26. The stages in the development of a monocyte are:

- Monoblast.
- Promonocyte.
- Monocyte.

11-27. *Monoblast.* This cell is extremely difficult to differentiate from the myeloblast. The nucleus is round or oval and appears more lightly stained than in the myeloblast. The nuclear chromatin is fine and delicate with abundant sharply defined, pale pink or blue parachromatin. One or two nucleoli are present. There is a moderate amount of basophilic or grey tinged cytoplasm. There are no granules in the cytoplasm.

11-28. *Promonocyte.* Some authorities do not differentiate the promonocyte from the monoblast. Descriptions sometimes differentiate the two cells by the presence of perhaps one nucleolus and very fine, lilac staining granules in the cytoplasm of the promonocyte. The granules are so small that they are called "azurophilic dust."

11-29. *Monocyte.* Mature monocytes are usually larger than other leukocytes in peripheral blood (15 to 25 microns). Unstained exhibit slow, ameoboid movement and may be seen on the stained slide with single or multiple pseudopods.

11-30. The nucleus of the monocyte is usually folded, but it may be round, kidney shaped, or deeply indented. One of the most distinctive features of the monocyte is the very fine, diffuse chromatin strands with abundant parachromatin in the nucleus. This diffuse, very light staining nucleus differentiates the monocyte from the lymphocyte and metamyelocyte. This delicate chromatin pattern is in contrast to the lymphocyte and metamyelocyte chromatin which is clumped. Nucleoli are absent. The cytoplasm of a monocyte is opaque, gray blue without the clear perinuclear zone described for most lymphocytes. It has been described as "foamy" or having a ground glass appearance. There is a large amount of cytoplasm in relation to the nucleus, which is also in contrast to the lymphocyte. The nonspecific, fine, azurophilic granules of the monocyte are dustlike and lilac staining. A few large, unevenly distributed azurophilic granules may be seen in overstained smears. If overstained, the monocyte may be confused with metamyelocytes.

11-31. Monocytes may be difficult to differentiate from other cells, particularly if the stain is not good. In poorly stained blood smears, the delicate nuclear morphology will appear less distinct and the coarse granules will confuse the picture. When other differential features are absent, the brainlike convolutions of the nucleus and the dull gray-blue color of the cytoplasm are usually sufficient to classify the cell as a monocyte.

11-32. The classification of normal blood cells into specific categories is sometimes difficult. Sometimes it is not possible to do so. While normal blood cells, as a rule, follow the patterns we have discussed, they don't always do so. If the technician becomes thoroughly familiar with the normal characteristics of a particular type of cell, he will be more aware of atypical cells when he sees them and will be better prepared to evaluate the abnormal cells that are considered in the following section.

12. Leukocyte Abnormalities

12-1. Blood cells may show distortion artifacts. Distorted cells occur as a result of compression or crushing. Compression is due to the pressure of cells upon each other, and crushing is by mechanical pressure which ruptures cells when the smear is made. Compressed cells appear smaller than normal with darker staining cytoplasm. Monocytes and lymphocytes are easily crushed. The monocyte, when crushed, may exhibit a U-shaped nuclear remnant. The lymphocyte will develop "spindle forms." Abnormal crushed cells are the "smudge" or "basket cells" which are immature or fragile leukocytes. These smudge cells are found predominantly in disease with an acute shift toward immature forms, e.g., leukemias.

12-2. The terminology used to designate increases or decreases in the various types of white cells is shown in table 7. A leukocytosis is present if the total circulating leukocyte count is above 11,000/cmm., and a leukopenia is present if the leukocyte count is less than 4,000/cmm.

12-3. A leukopenia due to a decrease in the number of neutrophils is specifically referred to as "neutropenia." A leukopenia also includes the lymphocytes and other white blood cells. A leukopenia, or count of 4,000 per cmm. due to the reduction in neutrophils, could show 50 percent lymphocytes.

12-4. An understanding of leukocytosis, leukopenia, and the differential leukocyte count is essential before studying the leukemias, which might be confused with diseases accompanied by leukocytosis. In leukemias there is an increase in the

TABLE 7
TERMINOLOGY APPLICABLE TO INCREASES OR DECREASES IN THE PRINCIPAL TYPE OF WHITE CELLS

Type of cell	*Increase termed*	*Decrease termed*
Neutrophil	Neutrophilia	Neutropenia
Eosinophil	Eosinophilia	Eosinopenia
Basophil	Basophilia	Basopenia
Lymphocyte	Lymphocytosis	Lymphocytopenia
Monocyte	Monocytosis	Monocytopenia

numbers of the abnormal and primitive cells in the peripheral blood as well as in the bone marrow.

12-5. In polycythemia vera, a benign disease which may be confused with leukemia, there is an absolute increase in all stages of the leukocytes, blood platelets, and erythrocytes. Histochemical studies of cells from cases of polycythemia vera indicate that the cells are probably normal. Sometimes in polycythemia vera, granulocytic leukemia develops.

12-6. Infections with pyogenic bacteria cause striking examples of neutrophilic leukocytosis. Localization of the infection with pus under tension may cause a severe leukocytosis. Then again, the infection may be so severe or overwhelming that leukocyte production is depressed rather than stimulated. Marked leukocyte responses occur in infancy and childhood, while in older individuals the changes are not as marked. Generally, acute infections cause a leukocytosis, while chronic infections may fail to stimulate production of leukocytes and are associated with a neutropenia. In infections due to viruses, there may be an initial neutrophilic leukocytosis. A relative or absolute lymphocytosis with neutropenia is usually present.

12-7. Neutrophilic leukocytosis is almost always due to a hyperplasia of certain cells in the bone marrow. The blood picture reflects the degree of production of these cells in response to the body need. A shift to the left in the differential count means that more immature cells are being liberated from the bone marrow due to an unusual demand. A shift to the right means more mature cells with some neutrophils with hyperlobation and more degenerative forms are present. In the presence of a neutropenia, it is indicative of reduced marrow production of neutrophils.

12-8. In leukemoid states, the blood picture may be that of a true leukemia. However, when a disease is accompanied by anemia, thrombocytopenia, splenomegaly, hepatomegaly, or lymph node enlargement, it may be difficult to distinguish the leukemoid reaction from leukemia. A leukemoid blood picture may involve either the granulocytic or agranulocytic series. A great increase in the leukocytes without extreme immaturity suggests a leukemoid reaction. However, a shift to the left so extreme as to show promyelocytes, and a rare "blast" form, may occur in leukemoid reactions. Also, achronic granulocytic leukemia may show few "blast" forms in the blood, and a bone marrow aspiration may be necessary to differentiate the two. Even the bone marrow study may not be diagnostic. Granulocytic leukemoid reactions most commonly occur in whooping cough, chicken pox, and infectious mononucleosis, and when bone marrow is replaced by metastatic tumor.

12-9. Infectious mononucleosis is a self-limiting disease thought to be caused by a virus. The disease affects children or young adults, and has an incubation period of 10 to 14 days. The onset is characterized by fever, sore throat, and enlarged cervical lymp nodes. There may be involvement of the whole body, especially the lympatic tissues of the reticuloendothelial system, with symptoms and signs varying with the organs and tissues involved.

12-10. Diagnosis of infectious mononucleosis is aided by microscopic examination of blood and the heterophil antibody test. Occasionally, a bone marrow biopsy may be necessary to rule out leukemia. Leukocytosis may be present in the early stages before enlargement of the lymph glands. However, the total leukocyte count may be normal or low. There is an increase of both lymphocytes and monocytes. These two types of cells usually average 60 percent of the total count. A differential count containing less than 40 percent agranulocytic cells usually does not indicate progressed infectious mononucleosis.

12-11. Atypical lymphocytes are characteristic of mononucleosis. These atypical lymphocytes or virocytes have been divided into three groups by Downey and McKinlay. This classification is less important today from a clinical viewpoint than it was a few years ago.

12-12. The classic Downey cells of infectious mononucleosis are probably the best examples of cells reported as atypical lymphocytes. This is not to say that they are the only ones. Other viral infections generate their full share of atypical cells, and very prominant among these are atypical lymphocytes. Downey's original description of the atypical lymps of infectious mononucleosis is of historical interest, but the classification by type is not applicable since there is no correlation of the types with the severity of the disease. Atypical lymphocytes, per se, are not pathognomonic of infectious mononucleosis. An atypical lymphocyte may be large or small. It may have vacuoles in the cytoplasm, in which case the cytoplasm has been described as "foamy" in appearance; or it may have scant or abundant cytoplasm that is deeply basophilic, staining deep blue; or, it may have both basophilic and vacuolated cytoplasm. If a lymphocyte contains definite nucleoli (lighter staining punched-out areas in the nucleus), it is always atypical, whether it contains basophilic, vacuolated cytoplasm or not. Those lymphocytes containing nucleoli may be misinterpreted as leukemia.

12-13. The distinguishing features of the great majority of atypical lymphocytes are vacuolated cytoplasm, deeply basophilic cytoplasm, and nucleoli in the nucleus. Any one of these three morphological characteristics or any combination of these is indicative of an atypical lymphocyte, and the percentage should be reported. If there is an absolute lymphocytosis, close observation of the individual cells should be made for atypical features.

12-14. The morphologic diagnosis is of value, but the final diagnosis may depend upon serological tests. These tests will be discussed in Volume 3 of this course. After the clinical condition subsides, the "atypical" cells may be seen for many months. The bone marrow picture of infectious mononucleosis is not constant in appearance and can be differentiated from leukemia.

12-15. Infectious lymphocytosis is a contagious disease of young children, believed to be caused by a virus. Clinical manifestations are mild or may even be absent. The leukocytosis may be as high as 50,000 per cmm., and sometimes over 100,000 per cmm. There is both an absolute and relative increase of normal, small lymphocytes. The lymphocytosis may last 3 to 5 weeks, or slightly longer. The bone marrow is not remarkable in the disease, for there is a lymphocytosis, but there may be a granulocytopenia.

12-16. Leukemia may be defined as a neoplastic disease characterized by the proliferation of hematopoetic cells in the bone forming organs and peripheral blood. The diagnosis should be made by a pathologist or hematologist. This is an extremely serious disease, and the treatment involves potent toxic drugs. The duration of the disease depends upon the type of leukemia and varies from a few days to as long as 20 years. The more common types of leukemia are discussed below.

12-17. Chronic granulocytic leukemia is characterized classically by a very high white count consisting of cells of the granulocytic series in various stages of maturation, usually with many mature forms. There is associated anemia. The platelets may be either decreased or marked by increase, giving rise to thrombotic episodes in some cases. This type of leukemia in many cases is difficult to differentiate from a leukemoid reaction even with a bone marrow aspiration. Chromosome and chromosomal studies may be quite helpful. Granulocytic leukemia is associated with chromosomal abnormality on the 21st chromosome. This is called the *Philadelphia chromosome*. The leukocyte alkaline phosphatase test is low in granulocytic leukemias and is a frequently used test to differentiate leukemoid reactions from leukemia.

12-18. Chronic lymphocytic leukemia is a disease that usually affects patients who are older than 50. It is associated with the best prognosis among the leukemias. Some patients live as long as 20 years with the disease. Patients may be asymptomatic and yet have a 100,000 white cell count. In these circumstances many physicians withhold treatment. The blood picture shows large numbers of mature lymphocytes, and the bone marrow, spleen, and lymph nodes are markedly affected. There is associated anemia, leukopenia, and thrombocytopenia.

12-19. The acute leukemias usually have a rapid course and are characterized by numerous blast cells in the peripheral blood. The types of acute leukemia are in many cases differentiated with difficulty. It is important, if at all possible, to differentiate granulocytic and lymphocytic varieties because the latter are more amenable to remission with present drug therapy than the former. In acute granulocytic leukemia, thin, eosinophilic, rod shaped structures are seen in the cytoplasm of an occasional cell. These are called Auer rods (bodies). These leukemias have associated profound anemia and thrombocytopenia. Other types of leukemia are rare and include monocytic, megakaryocytic, plasmacytic, and others.

12-20. As a final consideration in this chapter, we will briefly discuss the demonstration of L.E.

cells in patients with lupus erythematosus. An L.E. cell is shown in foldout 2. This disease is characterized by the presence of an abnormal plasma protein, which results in degeneration of nuclear material which then becomes phagocytized by other cells. This is demonstrated *in vitro* in the clinical laboratory by techniques with which you should already be familiar.

12-21. Free masses of lysed nuclear material, with or without polymorphonuclear leukocytes clustered about them (rosette formation), are suggestive of the L.E. phenomenon. Observing "rosettes" should encourage the technician to repeat the examination and search further for the true "L.E." cells. A positive report should not be made without conclusive identification of this cell. The inclusion body within the leukocyte is a homogeneous mass and has no chromatin pattern. This feature distinguishes the true "L.E." cell from the "tart" cell which contains a phagocytized nucleus. This latter cell contains an engulfed, damaged nucleus, usually that of a lymphocyte which still contains a recognizable chromatin pattern and a nuclear membrane.

MODIFICATIONS

Section 13
Pages 43-46 of this publication has(have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted materials are copyrighted and could not be duplicated for use in vocational and technical education.

14. Tests for Coagulation Deficiencies

14-1. When coagulation disorders are suspected, a number of tests are performed in order to detect or identify the cause of the disorder. Further, certain assay procedures are necessary to follow the course of therapy in conditions of known etiology. A prothrombin time is a good example of the latter. We will briefly discuss each of the major tests performed in the average clinical laboratory. Refer to table 9 as you read about each of these tests for an indication of their significance to the physician in evaluating coagulation disorders.

14-2. **Tourniquet Test.** In some bleeding disorders, capillary fragility is increased when the capillaries are subjected to increased positive pressure. This may be accomplished by placing a sphygmomanometer cuff on the arm of the patient. The cuff is inflated to a point midway between the stytolic and diastolic pressure. After 5 minutes the cuff is removed. One to two minutes after this the arm is examined for petechiae. The number of petechiae appearing on a representative 1 square centimeter area of the arm or back of the hand may be reported. Another method of reporting is to draw a circle about the size of a 25-cent piece

SCHEMATIC OF THE COAGULATION MECHANISM

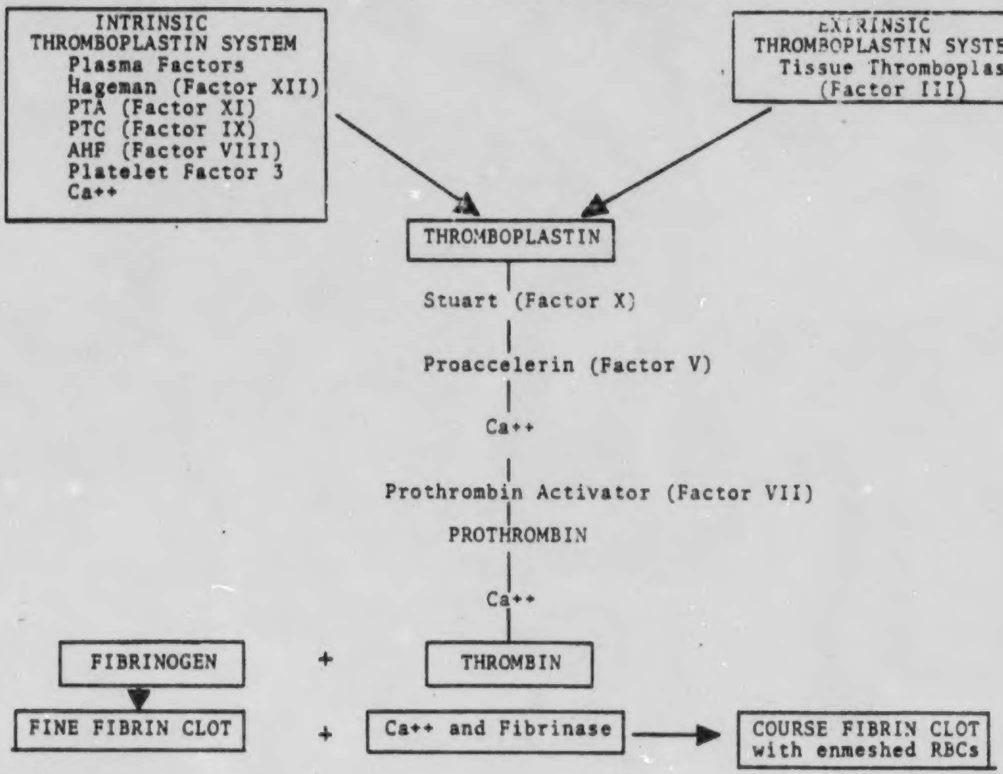
INTRINSIC THROMBOPLASTIN SYSTEM
Plasma Factors
Hageman (Factor XII)
PTA (Factor XI)
PTC (Factor IX)
AHF (Factor VIII)
Platelet Factor 3
Ca++
EXTRINSIC THROMBOPLASTIN SYSTEM
Tissue Thromboplastin (Factor III)
THROMBOPLASTIN
Stuart (Factor X)
Proaccelerin (Factor V)
Ca++
Prothrombin Activator (Factor VII)
PROTHROMBIN
Ca++
FIBRINOGEN
+
THROMBIN
FINE FIBRIN CLOT
+
Ca++ and Fibrinase
COURSE FIBRIN CLOT with enmeshed RBCs

Figure 11.

and to count the petechiae within the circle (0 − 10 = 1+; 10 − 20 = 2+; 20 − 50 = 3+; over 50 = 4+). Petechiae which appear only just below the cuff are not considered significant. In positive tests they appear all over the entire arm and hand.

14-3. Increased vascular fragility is sometimes found in qualitative and quantitative platelet abnormalities, vitamin C deficiency, dietary ascorbic acid deficiency, and in the various purpuras. The term "purpura" is not specific, but applies to a number of affectations characterized by bleeding into tissue. The tourniquet test is most often performed by the physician, and may be required in the clinical laboratory.

14-4. **Bleeding Time.** By definition, the bleeding time is the time required for a small standardized wound of the finger or ear lobe to stop bleeding. This test is very simple, but there is considerable variation in securing a "standardized" wound. In addition, some procedures recommend allowing the blood to flow freely, and others advocate blotting the blood as it accumulates. Blotting the blood often prolongs the bleeding time beyond the normal 3 minutes because blotting may remove small fibrin clots.

14-5. Bleeding time depends primarily on extravascular and vascular factors, and to a lesser degree on the factors of coagulation. Constriction of the blood vessels and action of the platelets are major forces in stopping the flow of blood. Some other variables in this test are skin temperature, circulation, the area punctured, and mechanical pressure applied to the wounded area.

14-6. **Whole Blood Clotting Time.** When blood is removed from a blood vessel and exposed to a foreign surface it clots. The clotting time can be altered by mechanical factors. Chief among these are temperature variations, test tube size, surface (coated or uncoated, wet or dry, smooth or rough), volume of blood in the tube, and the frequency as well as the way in which the tubes are tilted. The normal range depends upon how this test is performed. We suggest that you closely follow a recognized procedure such as the Lee-White clotting time outlined in AFM 160-51 and accept the normal values given for the particular procedure used. The normal range for nonsiliconized glass tubes is usually 5–10 minutes, but may run up to 15 minutes and still be considered normal.

14-7. Prolongation of the clotting time indicates a severe alteration of the coagulation mechanism. The abnormality may be due to *(a)* a defect in one or more stages of the coagulation process, *(b)* a deficiency of a specific clotting factor, or *(c)* the presence of an anticoagulant.

14-8. **Clot Retraction and Fibrinolysis.** Whole blood, having clotted, normally retracts from the sides of the container. This reaction is a function of the quantity and quality of intact platelets, the fibrinogen content of the plasma, and the ratio of plasma volume to red cell mass. Dissolution of the clot due to the action of proteolytic enzymes then ensues.

14-9. *Clot retraction procedure.* The standard procedure is to place a tube containing blood in a water bath and observe for retraction of the clot at the end of 1 hour and 2 hours. A semiquantitative technique may be performed by measuring the amount of serum expressed. This may be related to the total volume and reported in percent according to the formula:

$$\frac{\text{Volume of expressed serum}}{\text{Volume of whole blood}} \times 100$$

Normally, 45 to 60 percent of the serum is expressed from the clot, and this will occur within 1 to 2 hours.

14-10. Poor clot retraction occurs in thrombocytopenia, qualitative platelet deficiency, and in cases of an increase in the red cell mass. Clot retraction may appear to be increased in severe anemia and extreme hypofibrinogenemia, because of the formation of a small clot due to the relative increase in plasma volume.

14-11. *Screening test for fibrinolysis.* The process of clot dissolution, or *fibrinolysis,* is a necessary activity following clot formation. Once the clot has served its purpose, it must be removed. The mechanism of clot dissolution is very complex. Through a series of activators and precursors, plasminogen, a widely distributed globulin, is converted to *plasmin.* (Another term for plasmin is *fibrinolysin.*) Plasmin acts locally to enzymatically dissolve the clot. Several major coagulation defects relate to fibrinolysis.[2] Consequently, it is a

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[2] Winterobe, M. M., p. 96, *op. cit.*

TABLE 9
RESULTS OF LABORATORY TESTS IN VARIOUS COAGULATION DISORDERS

Coagulation Disorder	Coagulation Test									
	TT	BT	PT	PC	PTT	CT	CR	TGT	Plt. ct.	Fib.
Vascular defect	+	+		+				±		
Thrombocytopenia	+	±		+			+	±	+	
Thrombocytopathia	±	±		+			+	+		
Deficiency of:										
Prothrombin			+		+					
Factor V			+	+	+			+		
Factor VII			+							
Factor VIII				+	+	+		+		
Factor IX				+	+	+		+		
Factor X			+	+	+	+		+		
Factor XI				+	+	+		±		
Factor XII				+	+	+		±		
Hypofibrinogenemia			+			+	+			+
Presence of fibrinolysin										+

TT = Tourniquet test
BT = Bleeding time
PT = Prothrombin time
PTT = Partial thromboplastin time
+ = Abnormal
± = May be abnormal
PC = Prothrombin consumption
CT = Clotting time
CR = Clot retraction
TGT = Thromboplastin Generation test
Plt. ct. = Platelet count
Fib. = Fibrinogen assay

TABLE 10
TABLE OF PLASMA RATIOS FOR FIBRINOLYSIS SCREENING TEST

Tube	1	2	3	4	5	6
Normal Plasma (ml)	1.0	0.8	0.6	0.4	0.2	0
Patient's Plasma (ml)	0	0.2	0.4	0.6	0.8	1.0
Thrombin (ml)	0.1	0.1	0.1	0.1	0.1	0.1

matter of laboratory importance to evaluate fibrinolytic activity.

14-12. The fibrinolytic activity of a patient's plasma may be semiquantitatively measured by adding the test plasma to normal plasma. After clotting, the mixture is observed for zones of lysis. In performing this test you would prepare mixtures of patient's plasma, normal plasma, and thrombin as shown in table 10. The tubes are placed in a water bath and examined hourly for lysis. If necessary, incubation is allowed to continue overnight. In the presence of an abnormally high level of lysin, lysis will be observed within several hours. An approximation of lysin concentration may be determined by observing the ratio of patient to normal plasma. Tubes 1 and 6 are the normal and the patient's plasma controls, respectively. If the clots in all the tubes remain solid and well formed, the test is considered negative. Bacterial contamination must be avoided.

14-13. **Platelet Count.** For the accurate enumeration of platelets, the proper collection of blood is important. Unsatisfactory collection of the blood frequently results in platelet clumping; and when this occurs, the precision of the count is greatly reduced. Venipuncture must be made without difficulty, and the time between the withdrawal and the anticoagulation or dilution of the blood must be kept to a minimum. For best results, collect blood with a 20 gauge needle and a siliconized syringe. Transfer the blood to a small siliconized test tube which contains EDTA. Capillary blood from a clear finger puncture may be diluted directly. Nonwetable plastic equipment may also be used. A commercial kit is available which contains disposable pipettes and diluent. This kit is the *Unopette*, a registered trademark of Becton, Dickinson and Company, Rutherford, New Jersey.

14-14. *Direct methods.* You are already familiar with the standard method of counting platelets using Rees-Ecker diluting fluid. Rees-Ecker solution is an isotonic mixture which contains brilliant cresyl blue. This procedure is outlined in AFM 160-51, *Laboratory Procedures in Clinical Hematology*.

14-15. Most well equipped laboratories perform the direct platelet count by phase microscopy. Blood, drawn to the 1 mark in a RBC pipet, is diluted to the 101 mark with 1% ammonium oxylate. Shake for 3 minutes. It is important with phase microscopy that the diluent be optically clear; i.e., free from dirt, dust, etc. If such contaminants are present, they may be quickly removed by filtering the diluent through a membrane filter. Thin hemocytometers designed for the purpose are covered with a No. 1 coverglass instead of the standard hemocytometer coverglass. The hemocytometer is placed on wet filter paper and is covered with a petri dish cover for 15 minutes to allow the platelets to settle. Platelets are then counted. The necessary equipment consists of a long-working-distance condenser with a 43X annulus, a 43X phase objective (medium dark contrast), and a 10X ocular.

14-16. Platelets stand out as individual round or oval bodies with a pink or purple sheen. On focusing up and down, you can see one or more fine processes extending from the platelets. Crystals, dirt, and bacteria are readily distinguished by their increased refractility, angular shapes, and the absence of pink-purple sheen. The platelets in the four corner squares and the central square (as for red cell counts) are counted in each chamber. If the number of platelets counted in the total of 10 squares is less than one hundred, additional squares should be examined until a total of at least 100 platelets have been counted. If the total number of cells in 50 small squares (25 on each chamber) is less than 50, the count should be repeated, making an initial dilution of 1:20 in a white cell pipette. If with the 1:20 dilution the number of platelets counted in 10 squares (5 on each side) is less than 100, additional squares should be examined until at least 100 platelets have been counted or until the platelets in all 50 squares have been enumerated. The calculation is as follows:

$$\frac{\text{No. of platelets counted} \times \text{dilution}}{0.004 \times \text{No. of squares counted}} = \text{No. per cu mm}$$

14-17. Platelet counts by this method are considerably more accurate than by the Rees-Ecker method. The method of blood collection reduces platelet agglutination, fragmentation, and disintegration. The use of ammonium oxalate diluent clears the background by hemolysis of the red cells. The use of phase contrast microscopy overcomes the difficulty in distinguishing platelets from extraneous particles. Technically, the method is less difficult and time consuming than the less accurate Rees-Ecker method. The only disadvantage is that it requires specialized equipment. For those laboratories that are equipped, or that can be equipped, with a phase contrast microscope, this method is strongly recommended. The following precautions should be observed:

a. The presence of platelet clumps in the hemocytometer chamber indicates that incipient clotting has occurred. This may result from difficulties in entering the vein, delay in anticoagulating the blood, or delay in diluting the blood with ammonium oxalate solution when capillary blood is diluted directly. The presence of platelet clumps precludes reliable counts, and a fresh sample must be collected.

b. If the pipettes are allowed to rest for even 10 seconds after shaking, they must be reshaken before loading the chambers.

14-18. *Indirect thrombocyte count.* Thrombocytes are counted indirectly on a stained blood smear and expressed in number per 1,000 red blood cells. The number of thrombocytes per cu mm can be easily estimated if you know the red blood cell count and how many thrombocytes there are per 1,000 red blood cells:

$$\frac{\text{RBCs/cmm} \times \text{thrombocytes per 1,000 RBCs}}{1000} = \text{thrombocytes per cu mm}$$

If fewer red blood cells are counted, the formula is adjusted accordingly, with some loss of accuracy. This method should not be attempted without some type of ocular disc to narrow the field of vision.

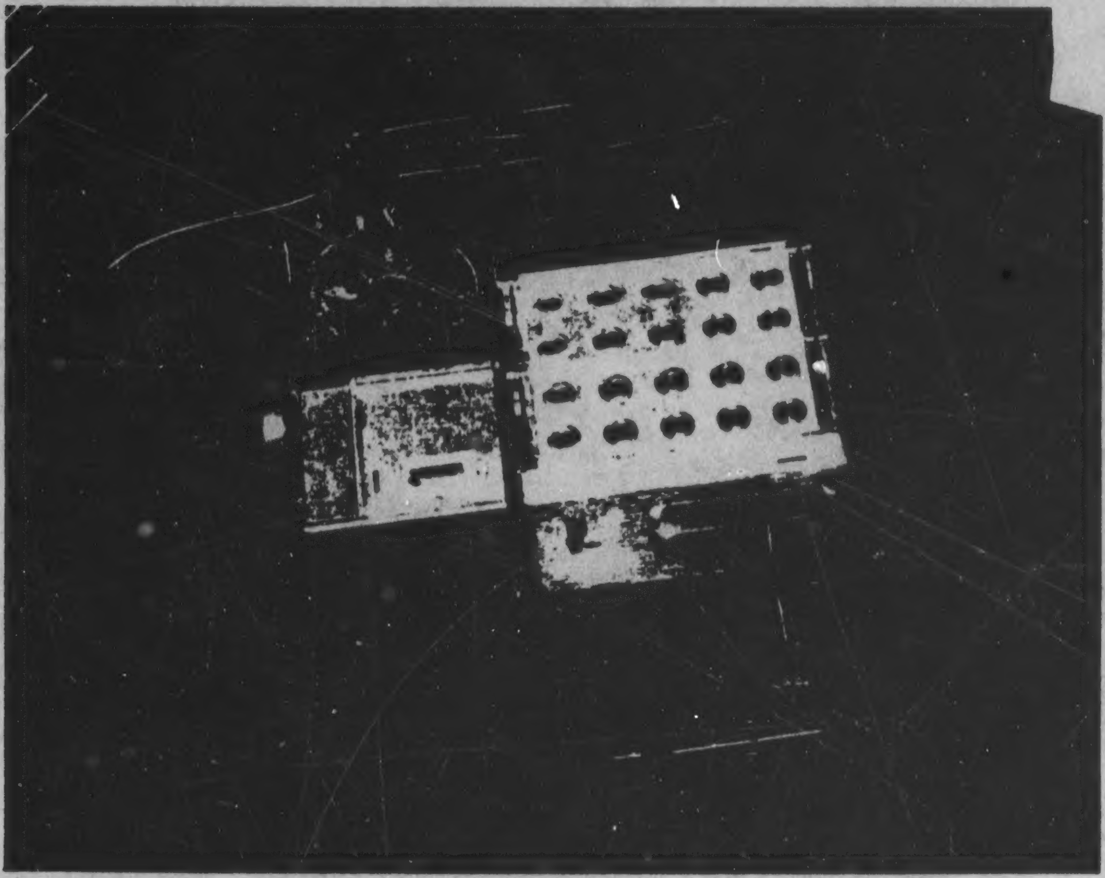

Figure 12. Fibrometer.

14-19. **Fibrinogen Estimation.** There are various means of estimating fibrinogen levels, none of which is completely reliable. The method presented in AFM 160–51 involves diluting control samples of diagnostic plasma to a fibrinogen content of 125 mg percent or less. These controls are clotted with *Simplastin*[3] and compared with the patient's plasma which is also clotted with *Simplastin*. Much simpler methods for estimating fibrinogen levels are available in kit form. One popular method involves diluting a drop of blood by finger puncture directly into a buffer solution. The diluted blood is reacted on a card or slide and compared with a control. This appears to be a convenient means of indicating decreased fibrinogen levels.

14-20. **Prothrombin.** In the presence of excess tissue thromboplastin and an optimal calcium level, plasma clots rapidly (11–15 seconds). The time required for clotting is known as the one-stage prothrombin time. The prothrombin time of a patient's plasma may be expressed as a percentage of the control plasma and reported as *percent activity*. However, the current trend is away from reporting percent activity. A commercial control should be used, not a so-called "normal" patient. If a locally prepared control must be used, pool three to five normal individuals who are not undergoing treatment.

14-21. In the Quick method of prothrombin testing, 0.1 ml. of plasma to be tested is mixed with 0.2 ml. of commercially prepared thromboplastin. The amount of time that elapses prior to a clot being formed is the prothrombin time. The length of time required is determined by tilting the tube at selected intervals or by using a wire loop to entangle the clot. The first appearance of a clot is the end point of the test. The Quick prothrombin time is very adaptable to testing with automatic timing devices. One such instrument is illustrated in figure 12. An automatic dispensing pipette that dispenses both patient speciment and thromboplastin is seen at the bottom of the picture. The main unit in the picture is composed of two components: a series of incubator wells on the right and the actual timing device with more incubator wells on the left. Preincubated patient plasma is added to a plastic cup in the well under the center post seen just above the electrical socket. The automatic dispenser is then filled with preincubated thromboplastin. The dispenser is reset so that the clot timing device is actuated when the plunger is depressed. When the clot timer is actuated, the center post drops down so that a wire loop and a wire needle come into contact with the plasma-thromboplastin mixture. When enough of a clot has formed to simultaneously contact both loop and needle, the timing device stops. The prothrombin time is read directly from the digital readout register on the lower end of the timing device. Due to wide variations in individual technique, the use of an automatic instrument to perform prothrombin times is more reliable than doing the test with test tubes, pipettes, and a stop watch. This is especially so if several technicians perform these tests on a rotating schedule.

14-22. Normal plasma has a prothrombin time of 11 to 15 seconds, depending on the activity of the thromboplastin. The clotting time of the normal plasma represents 100 percent activity for the system on a particular day. The daily control should always have a 100 percent value that is within the range of the normal samples used to construct the curve. The prothrombin time is prolonged if there is a decrease in the vitamin K-dependent clotting factors of the second stage of coagulation, namely prothrombin and Factors VII (stable) and X (Stuart). A decrease in Factor V (labile) or a fibrinogen (Factor I) concentration of less than 80 mg. percent will also prolong the prothrombin time.

14-23. The one-stage prothrombin time is misnamed because it is not a measure of prothrombin activity alone. It also reflects the activity of other second and third stage clotting factors. For this reason, an unexplained prolonged prothrombin time necessitates an assay of other clotting factors. Some constituents, e.g., Factors VIII and IX, are reported to have no significant effect on the one-stage prothrombin time. In spite of some interdependence among factors, the one-stage prothrombin time is still the most widely used and valuable test to monitor anticoagulant therapy with thrombinopenic drugs.

14-24. There are certain precautions to observe when performing a prothrombin time test. Plasma should be examined as soon as possible following collection. Standing either at room temperature or in the refrigerator for more than a few hours results in loss of Factor V. There is recent evidence that Factor V destruction is hastened by the use of 0.1M sodium oxalate as the decalcifying agent. For this reason, it may be preferable to use 3.8 percent sodium citrate as the anticoagulant.[4] Precautions other than storage time include temperature control and the use of suitable thromboplastin extract. Be particularly careful in reconstituting the thromboplastin preparation to avoid loss of the dry powder when adding the water. The reconstituted reagent is stable for 48 hours at refrigerator temperature. Write the time and date on the label to indicate when the thromboplastin was reconstituted. This will help prevent the use of unsuitable reagent. It is possible to divide the reconstituted thromboplastin mixture into aliquots and store in the frozen state for a month. However, it must be quick frozen and stored at 0 to 20° C., and aliquots must not be thawed and refrozen.

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[3] *Simplastin*. Registered Trademark of General Diagnostics Division, Warner-Chilcott.

[4] Barrington, J. D., Peterson, E. W., *Laboratory Control of Anticoagulation Therapy*. USAF School of Aerospace Medicine June 1967.

14-25. **Prothrombin Consumption Test.** As blood clots, prothrombin is converted to thrombin. The clotting factors that are necessary for the formation of intrinsic thromboplastin must be present in the blood in adequate amounts for this consumption of prothrombin to occur as the clot forms. One measure of the efficiency of the clotting mechanism is the amount of prothrombin that remains after clotting has occurred. Since fibrinogen and Factor V are also consumed when clotting occurs, they must be furnished by adding barium sulfate-adsorbed plasma which contains fibrinogen and Factor V, but no prothrombin or Factor VII. Barium sulfate-adsorbed plasma can be prepared from normal plasma or procured commercially. A technique for this test is found in AFM 160–51. However, AFM 160–51 suggests adding fibrinogen rather than $BaSO_4$-adsorbed plasma. This is an error which will probably result in a false-normal test because Factor V is not available and the mixture will not clot.

14-26. If properly performed, the prothrombin time from serum fortified with barium sulfate-adsorbed plasma should be more than 30 seconds. Results are considered valid only if the one-stage prothrombin time is normal. The prothrombin consumption test is abnormal in platelet deficiencies of the thromboplastin precursors (Factors VIII, IX, X, XI, and XII). It is important that the barium sulfate-adsorbed plasma be deprothrombinated sufficiently to give a prothrombin time over 1 minute when checked with the thromboplastin preparation.

14-27. **Partial Thromboplastin Time.** Certain thromboplastins are unable to compensate completely for the defect in hemophilic diseases. These substances are termed "partial thromboplastins." Partial thromboplastin is more sensitive to a decrease in plasma factors and gives a normal plasma clotting time (12–15 seconds). Several partial thromboplastin preparations are available commercially. These include *Potelin,*[5] *Thrombofax,*[6] and *Asolectin.*[7] The first two are brain cephalin products and the third is a soy phospholipid extract. As usual, follow the manufacturer's instructions when using products of this type.

14-28. The usual technique is to add 0.1 ml. of 0.025M calcium chloride to a 1:1 mixture of patient's plasma and partial thromboplastin. Normal range should be established with normal plasma. If the patient's plasma is abnormal, mixed studies may be performed as a screening differential diagnostic clue. This is done by labeling three tubes *a*, *b*, and *c*. To each tube add 0.1 ml. partial thromboplastin and incubate at 37° C. To tube *a*, add 0.1 ml. of a 1:1 mixture of normal plasma and patient's plasma. To tube *b*, add 0.1 ml. of a 1:1 mixture of fresh barium sulfate-adsorbed plasma and patient's plasma. To tube *c*, add 0.1 ml. of a 1:1 mixture of known deficient plasma and pa-

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[5] Warner-Chilcott, Morris Plains, N. J.
[6] Ortho Pharmaceutical Co., Raritan, N. J.
[7] Associated Concentrates Co., Woodside, N. Y.

TABLE 11

RESULTS OF THE THROMBOPLASTIN GENERATION TEST IN VARIOUS DISORDERS

Coagulation abnormality	Reagent		
	Adsorbed plasma	Serum	Platelets
Factor V	Abnormal	Normal	Normal
Factor VII	Normal	Normal	Normal
Factor VIII	Abnormal	Normal	Normal
Factor IX	Normal	Abnormal	Normal
Factor X	Normal	Abnormal	Normal
Factor XI	Abnormal	Abnormal	Normal
Factor XII	Abnormal	Abnormal	
Inhibitor	Abnormal	Abnormal	Normal
Thrombocytopathia	Normal	Normal	Abnormal

tient's plasma. You would, of course, proceed to add calcium chloride and determine the clot time for each tube. If tube *a* is normal, this rules out an anticoagulant. If *b* is also normal, this suggests Factor VII deficiency; if *b* is not normal, Factor IX deficiency is a possibility. If *c* is normal, the plasmas have different deficiencies; however, if *c* is not normal, the plasmas have the same deficiency.

14-29. The partial thromboplastin time is prolonged in deficiencies of prothrombin and Factor V, as well as in deficiencies of all the plasma factors in the intrinsic system. To exclude an abnormality of a factor in the extrinsic system, a prothrombin time test should be performed on all abnormal plasmas.

14-30. **Thromboplastin Generation Test.** The combination of adsorbed plasma, serum, platelets, and calcium forms a mixture capable of generating a potent thromboplastin. The efficiency of this mixture may be measured according to the ability of the thromboplastin to clot a plasma substrate. Technique for the thromboplastin generation test may be found in almost any current manual of blood coagulation methods. This procedure does not appear in AFM 160-51 (April 1962), however. Since one of the reagents required is platelet rich plasma, siliconized glassware must be used. (Refer to the appendix for reagent preparation.)

14-31. Results of the thromboplastin generation test are interpreted in terms of each of the reagents used; i.e., substrate plasma, adsorbed plasma, serum, and platelets. A defect in the adsorbed plasma indicates a Factor V or Factor VII abnormality. If Factor V is decreased, the prothrombin time should be prolonged. An abnormality in the serum fraction suggests a Factor IX or X deficiency. If Factor X is decreased, the prothrombin time should be prolonged. Finally, if the adsorbed plasma and serum are abnormal, Factors XI and XII are considered. Failure to obtain any degree of correction when normal reagents are substituted may indicate an inhibitor directed against thromboplastin formation. These results are listed in Table 11.

14-32. There are many additional tests which may be performed to identify coagulation defects. We have presented the most common and reliable tests that may be performed in any well equipped laboratory. As you can readily see, the concept of blood coagulation is by no means simple; neither are the tests and interpretations required to accurately identify every possible coagulation problem. Unusual or difficult areas are most effectively pursued at large facilities which are staffed with specially qualified hematology technicians. In some cases, the patient must be referred to such facilities because a complete laboratory evaluation cannot be performed on blood that is delayed by transportation.

APPENDIX

Preparation of Blood Reagents for Coagulation Studies

APPENDIX

PREPARATION OF BLOOD REAGENTS FOR COAGULATION STUDIES

1. Substrate plasma. Obtain blood samples from normal controls and patients using silicone-coated syringes (30 ml. is sufficient for the test). Add 9.0 ml. of blood to 1.0 ml. of either the citrate or oxalate anticoagulant. Centrifuge the blood at 2,500 rpm for 10 minutes, remove the plasma, and store it at 4° C. until ready to use. The substrate plasma serves as the source of prothrombin, Factor VII, and fibrinogen.

2. Deprothrombinized plasma. Mix normal and patient's plasmas each with a prothrombin-adsorbing reagent. Dilute the plasma 1:5 in saline and allow to stand for 1 hour, at 4° C., or in an ice bath before use. The adsorbed plasma is the source of Factors V, VIII, XI, and XII.

3. Platelets. Add 9.0 ml. of blood to each of two silicone-coated tubes containing 1.0 ml. of sodium citrate (sodium oxalate should not be used). Centrifuge at 1,000 rpm for 10 minutes or 2,500 rpm for 3 minutes. Remove the platelet-rich plasma with a silicone-coated pipette and record the volume. Transfer the plasma to a second silicone-coated tube and centrifuge at 2,500 rpm for 20 minutes. (The resultant platelet-poor plasma may be used for substrate, for adsorption, or for plasma factor assays.) Loosen the packed platelet button gently with an applicator stick, wash with saline by thorough mixing, and centrifuge. The washing procedure should be repeated a second time. Then suspend the platelets in an amount of saline equal to one-third the original plasma volume.

4. Barium sulfate adsorbed-plasma. Use chemically pure powdered barium sulfate, approximately 100 mg. of barium sulfate for each milliliter of plasma. Incubate the plasma—barium sulfate mixture for 10 minutes at 37° C. with frequent mixing. Certifuge the material and remove the upper three-quarters of the plasma. If properly adsorbed, the plasma should have a one-stage prothrombin time greater than 1 minute.

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ECI Form No. 17

STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume.

2. *Use the Guide as you complete the Volume Review Exercise and for Review after Feedback on the Results.* After each item number on your VRE is a three digit number in parenthesis. That number corresponds to the Guide Number in this Study Reference Guide which shows you where the answer to that VRE item can be found in the text. When answering the items in your VRE, refer to the areas in the text indicated by these Guide Numbers. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed.* Go to your VRE booklet and locate the Guide Number for each item missed. List these Guide Numbers. Then go back to your textbook and carefully review the areas covered by these Guide Numbers. Review the entire VRE again before you take the closed-book Course Examination.

3. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the questions missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Number	*Guide Numbers 100 through 116*
100	Introduction to the Physiology of Blood; The Composition of Blood, pages 1-3
101	Functions of Blood: General; Oxygen-Carrying Capacity, pages 3-6
102	Functions of Blood: Erythrocyte Metabolism; Leukocyte Functions; Circulation, pages 6-9
103	Introduction to the Complete Blood Count and Related Studies; Collecting a Blood Sample, pages 10-12
104	Cell Counts, pages 12-17
105	Microscopic Studies, pages 17-21
106	Hemoglobin and Hematocrit Studies, pages 21-25
107	Statistical Analysis, pages 25-27
108	Introduction to Erythrocyte Studies; Morphology of Erythrocytes, pages 28-31
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110	Evaluation of Red Cell Abnormalities, pages 33-35
111	Introduction to Leukocyte and Thrombocyte Maturation; Normal Maturation Sequence, pages 36-39
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116	Appendix: Preparation of Blood Reagents for Coagulation Studies, pages 55-57

CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 1

Objectives: To be able to recognize the principal cellular and plasma constituents of blood, together with their related functions; and to be able to outline the physiology of respiration with reference to the oxygen-carrying capacity of blood.

1. Where are your red blood cells produced? (1-7, 8)

2. (a) Define mitosis; meiosis. (b) Which of these two terms applies to the production of blood cells? (1-9)

3. Where are most of your white blood cells produced? (1-10)

4. The normal RBC has a *half-life* of 28 to 38 days. What does this mean? (1-14)

5. List some of the functions of blood. (2-3)

6. Distinguish between internal respiration and external respiration. (2-6)

7. What effect does water vapor have on air pressure in the alveoli? (2-8)

8. At which point will oxygen cease to diffuse into the capillaries surrounding the alveolus? (2-9)

9. (*a*) Of what physiological significance is the partial pressure of carbon dioxide in venous blood as compared with the partial pressure of carbon dioxide in the alveolus?
   (*b*) What are the values for these partial pressures respectively?
   (2-9, 10)

10. How do you explain the fact that CO_2 diffuses readily between the venous blood and alveoli with a pressure gradient of only 6 mm Hg, whereas oxygen requires a much higher differential gradient to diffuse into the cappillaries? (2-10)

11. (*a*) What is the physiological difference between arterial and venous blood?
    (*b*) Do all arteries contain arterial blood in the physiological sense? Explain your answer.
    (2-14)

12. (*a*) Briefly describe the hemoglobin molecule. (2-15)
    (*b*) How does the hemoglobin molecule "carry" oxygen? (2-17)

13. (*a*) What is the difference between a microphage and a macrophage?
(*b*) Give an example of each.
(2-20, 21)

14. What is current thinking regarding the function of lymphocytes? (2-24)

15. (*a*) Outline the course of blood through the pulmonary circulatory system.
(*b*) Outline the course of blood through the systemic circulatory system.
(2-28, 29)

16. You perform a hemoglobin on a patient who is bleeding quite severely. The result is 11 g%. Thirty minutes later the hemoglobin value is still 11 g%. How do you explain this? (2-31-33)

CHAPTER 2

Objective: To show a knowledge of the principles and procedures involved in a complete blood count and semen analysis.

1. (*a*) What is the primary advantage of a venipuncture over a capillary puncture?
(*b*) What is the primary disadvantage of the venipuncture?
(*c*) Is there any significant difference between capillary blood and venous blood with respect to routine hematology results?
(Intro.-2)

2. Excluding infants, describe specifically the best site for a capillary puncture for most studies. (3-3)

3. (*a*) What technique might you use to obtain blood for a CBC and microbilirubin from an infant 3 days old?
   (*b*) What precaution should you exercise when employing this technique?
   (3-3)

4. Exactly what do we mean by an *atraumatic* venipuncture? (3-5)

5. Under what circumstances, if any, may you draw blood from a femoral vein? a jugular vein? (3-6)

6. In what type of blood work is the time of tourniquet application particularly critical? (3-6)

7. How would you describe the action of 70 percent isopropyl alcohol as a venipuncture site cleanser? (3-8)

8. What should you do if blood infiltrates and the tissue "wells up" around a venipuncture site while you are drawing blood? (3-9)

9. What is a possible objection to merely bending the elbow as a post-venipuncture posture? (3-10)

10. (*a*) Which anticoagulant has the least effect on the size of cellular components?
(*b*) What is the anticoagulant of choice for routine CBCs?
(*c*) What is the objection to using heparin routinely for CBCs?
(3-12, 13)

11. (*a*) If you draw blood to the 0.3 mark in an RBC pipette and dilute to the 101 mark, what is the dilution?
(*b*) When might this particular dilution be of practical value?
(4-7)

12. In the manual routine RBC count, what is the factor of (*a*) dilution, (*b*) depth, (*c*) area? (4-10)

13. In the manual routine WBC count, what is the factor of (*a*) dilution, (*b*) depth, (*c*) area? (4-13)

14. In performing a manual routine total cell count or CSF, you count 9 cells in the undiluted specimen. What is the reported cell count in number per cmm.? (4-15)

15. Or what general value is a CSF differential to the clinician? (4-17)

16. Assuming that a semen specimen is collected in the hospital, at which time intervals following collection should you examine it for motility? (4-21)

17. (*a*) List the ingredients recommended in the text for diluting semen.
(*b*) What effect, if any, does this diluent have on the spermatozoa?
(4-22)

18. (*a*) You mix 0.1 ml of semen with 1.9 ml of diluting fluid, and you perform the count as you would a routine RBC count. If you count 127 cells, what is the reported count in number per ml?
(*b*) What is the normal for a sperm count?
(4-22)

19. Briefly describe the principle of:
(*a*) The optical system for electronic cell counts.
(*b*) The resistance type counter.
(4-23)

20. List the most common sources of error in electronic cell counting. (4-27)

21. (*a*) How can you determine the "safe period" in which WBC counts may be performed following addition of the stromatolyzer?
(*b*) How long after addition of the stromatolyzer does dissolution of WBC's *usually* occur?
(4-28)

22. List the recommended daily checks to reduce instrument failures with electronic cell counters. (4-30)

23. To become proficient in performing differentials, what must all technicians be able to do? (5-4)

24. What is the best way to determine cell size? (5-9)

25. What do we mean if we say that a cell has an N:C ratio of 1:2? (5-12)

26. How is a band cell distinguished from the (*a*) metamyelocyte and (*b*) neutrophil? (5-14)

27. What is the most distinctive feature of an immature nucleus? (5-15)

28. How would you fix unstained blood smears which are to be held for more than 4 hours? (5-21)

29. What is probably the best action to take if a stain is too acid, with loss of detail to leukocytes other than eosinophils? (5-26)

30. How many platelets would you expect per oil immersion field on a normal differential smear? (5-29)

31. (*a*) What is the maximum probable error for spectrophotometric hemoglobin determinations according to the text?
(*b*) What is the most significant contributing factor to hemoglobin errors?
(6-4)

32. List some sources of error in the cyanmethemoglobin procedure. (6-9)

33. What is the principal objection to the oxyhemoglobin procedure as a manual method? (6-10)

34. (*a*) Name three "inactive" forms of hemoglobin.
(*b*) Why are they referred to as "inactive"?
(6-12)

35. List some sources of error in the microhemotocrit procedure. (6-14, 15)

36. Describe two ways by which the standard deviation concept is used for control and statistical evaluation in hemotology. (7-5, 8)

CHAPTER 3

Objective: To be able to describe the origin and maturation of erythrocytes; the various kinds of blood cell abnormalities; and the significance of various tests designed to measure cell function, size, shape, and count.

1. Name two qualitative variations which may occur in red cells. (Intro.-4)

2. Define erythropoiesis. (8-1)

3. How does the biconcave disk shape of the erythrocyte serve the function of the cell? (8-2)

4. Name the cell that follows the rubriblast in the red cell maturation series. (8-3)

5. What cell in the erythrocyte series can be described as being moderately large, with dark royal blue cytoplasm, a round nucleus, indistinct parachromatin, and distinct nucleoli? (8-4)

6. Name two morphological characteristics which distinguish the rubricyte from other cells in the erythrocyte series. (8-6)

7. What cell is normally the smallest, nucleated cell in the red cell series? (8-7)

8. How do reticulocytes differ from erythrocytes with diffuse basophilia or basophilic stippling? (8-8, 9)

9. Why does a normal, mature, red blood cell stain less intensely in the center? (8-11)

10. Normally, what can be said concerning the rate of development between the nucleus and cytoplasm in erythropoiesis? (8-12)

11. What is asynchronism? (8-13)

12. Name three morphological variations in red blood cells which might develop from genetic inheritance. (8-14)

13. What causes hypochromia? (8-16)

14. How the terms "polychromasia" and "diffuse basophilia" synonymous? (8-10, 17)

15. Describe a spherocyte. (8-18)

16. What would the observation of sickle cells mean if reported on a routine differential smear? (8-21)

17. What term describes the blue, threadlike figure eight rings found in red cells of patients with severe anemias? (8-24)

18. List three terms used to describe residual nuclear material in an erythrocyte. (8-23, 24)

19. What is a siderocyte? (8-27)

20. How do acanthocytes differ from spherocytes? (8-18, 28)

21. What is polycythemia? (9-2)

22. Name the three types of polycythemia? (9-3-5)

23. What factor concerning RBCs, other than a decrease in quantity, may indicate anemia? (9-6)

24. Define two terms used to describe the amount of color in stained erythrocytes? (9-7)

25. List three factors upon which normal hematopoiesis depends. (9-8)

26. Define hemoglobinopathy. (9-9)

27. Name two inherited hemoglobinopathies. (9-9)

28. How does free hemoglobin, released from the red cells by hemolysis, affect oxygen metabolism? (9-11)

29. What term is used to describe a hemolytic anemia manifested by abnormal cellular morphology abnormal hemoglobin, or both? (9-11)

30. How do antibodies cause hemolysis? (9-13)

31. What tests determine the elasticity or strength of the cell membrane? (9-17)

32. What characteristics would you expect to see in red cells of a microcytic hypochromic anemia? (8-14, 16; 9-18)

33. State whether the anemia resulting from hemolytic disease of the newborn is a congenital or acquired anemia and give the reason for your answer. (9-11-13, 19)

34. Name two possible known causes for aplastic anemia. (9-24)

35. What effect does reduced oxygen tension have on red blood cells containing hemoglobin "S"? (10-2)

36. What report is rendered for a sickle cell preparation. (10-3)

37. How is the erythrocyte sedimentation rate used in modern clinical medicine? (10-4)

38. How is the mass of a particle falling in solution related to its rate of fall. (10-5)

39. In considering the basic principles of erythrocyte sedimentation, what three circumstances increase the mass? (10-6)

40. Identify four technical aspects that influence erythrocyte sedimentation rate. (10-10-12)

41. What factor exerts the most clinically significant effect upon erythrocyte sedimentation? (10-13)

42. List three values which comprise the red cell indices. (10-14)

43. What three values must be determined for calculation of RBC indices? (10-15)

44. What precaution must be observed if an electronic particle counter is used to count the RBCs for indices calculation? (10-17)

45. What physical characteristic of a fluid affects the distortion or lysis of RBCs in that fluid? (10-18)

46. Identify the first observation made when an erythrocyte starts to lyse. (10-19)

47. Why is a red blood cell *not* lysed in isotonic (0.85%) saline? (10-20)

48. What may be done to increase the sensitivity of the RBC osmotic fragility test? (10-22)

CHAPTER 4

Objective: To demonstrate a knowledge of hemopoiesis and sites of cell production; the maturation sequence of white blood cells; the morphological characteristics of white blood cells; and the features of abnormal cells found in peripheral blood smears.

1. List four general trends observed in the maturation of all blood cells (11-1)

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2. What granules are normally observed first in the maturation of granulocytes? (11-2, 3)

3. Name the cell stages in the normal maturation order of the granulocytic series. (11-3)

4. Give the most distinguishing morphological characteristics of each of the following:
   (*a*) Myeloblast.
   (*b*) Promyelocyte.
   (*c*) Myelocyte.
   (*d*) Metamyelocyte.
   (11-4-10)

5. Why are eosinophilic and basophilic leukocytes not differentiated after the myelocyte stage of development? (11-13, 15)

6. What are distinguishing morphological characteristics of the megakaryocyte? (11-20)

7. Primarily, where do lymphocytes originate in the body? (11-21)

8. With reference to the morphological characteristics listed below, describe a lymphocyte:
   (*a*) Cell size.
   (*b*) Quanity of cytoplasm.
   (*c*) Staining or histochemical appearance of cytoplasm.
   (*d*) Cytoplasmic granules.
   (*e*) Shape of the nucleus.
   (*f*) Chromatin appearance.
   (11-25)

9. In considering morphology, what other leukocyte might be easily mistaken for a myeloblast? (11-27)

10. Name one of the most distinctive morphological features of the monocyte. (11-30)

11. Name two terms used to describe leukocyte remnants in a differential resulting from mechanical pressure. (12-1)

12. State the terms used to describe an increase or decrease in leukocyte number. (12-2)

13. How does the classification by type of atypical lymphocytes correlate with the severity of the disease? (12-12)

14. List three distinguishing, atypical, morphological characteristics observed in the great majority of atypical lymphocytes. (12-13)

15. Which hematology test is frequently used to differentiate a leukemoid reaction from granulocytic leukemia? (12-17)

16. Which of the acute leukemias is more amenable to remission? (12-19)

17. What are Auer rods (bodies)? (12-19)

18. Describe the difference between a true L.E. cell and a tart cell. (12-21)

CHAPTER 5

Objectives: To demonstrate an understanding of the concept of hemostasis, including both the intrinsic and extrinsic mechanisms; to be able to describe the principal tests used to follow bleeding disorders and anticoagulation therapy.

1. Define (*a*) hemostasis, (*b*) extravascular mechanism, (*c*) vascular mechanism, and (*d*) intravascular mechanism. (13-2)

2. Briefly list the three stages of blood coagulation. (13-5)

3. (*a*) In the intrinsic system, where are the factors responsible for coagulation found?
(*b*) In the extrinsic system, what is derived from the tissues?
(13-6, 7)

4. What does the partial thromboplastin time indicate? (13-14)

5. (*a*) Which stage of the clotting mechanism is measured by the prothrombin time test?
(*b*) Which factors does the test measure?
(13-18)

6. (*a*) What is the difference between hemophilia A and hemophilia B?
(*b*) Why is hemophilia more common in males than in females?
(13-21)

7. Which of the tests discussed in section 4 is most likely to indicate a deficiency of ascorbic acid in the diet?
(14-2, 3)

8. Is the bleeding time test generally a good screening test? Why or why not? (14-4)

9. What primary deficiency is measured by the clot retraction test? (14-10)

10. How can a phase microscopist differentiate platelets from debris? (14-15, 16)

11. What is the greatest potential source of error to the platelet in shaking the pipette and charging the chamber? (14-17)

12. List some precautions you should observe in performing the prothrombin time test. (14-24)

13. (*a*) Which stage of the clotting mechanism is measured by the prothrombin consumption test?
(*b*) Which test, if any, must be normal for the prothrombin consumption test to be valid?
(14-25, 26)

14. (*a*) Which factors, if deficient, will prolong the partial thromboplastin time?
(*b*) Which test would you be most likely to perform next if the partial thromboplastin time is abnormal?
(14-29)

15. Assuming that you have properly performed the test, what is suggested if you fail to obtain a degree correction when normal reagents are substituted in the thromboplastin generation test? (14-31)

ANSWERS FOR CHAPTER REVIEW EXERCISES

CHAPTER 1

1. In the red marrow of the so-called spongy bones. (1-7, 8)

2. (*a*) Mitosis is cell division without reduction in the number of chromosomes. Meiosis is reduction division in which sperm and egg cells develop with onehalf the number of chromosomes of their parent cells.
(*b*) Mitosis. (1-9)

3. In the bone marrow. (1-10)

4. This means that 28 to 38 days is required for one-half the number of cells tagged with a radioactive isotope to disappear from circulation. (1-14)

5. To transport oxygen and carbon dioxide; serve as an environment for body cells; regulate body temperature; distribute chemical substances; transport waste products; and maintain acid-base balance. (2-3)

6. Internal respiration is the exchange of oxygen and carbon dioxide between the blood and body cells. External respiration is the exchange of gases between the blood and alveoli of the lungs. (2-6)

7. Air pressure is reduced by the pressure of the water vapor. (2-8)

8. At the point where the alveolar pressure reaches 100 mm. Hg., which is also the partial pressure of oxygen within the capillary. (2-9)

9. (*a*) The difference between the two partial pressures results in an exchange of carbon dioxide into the alveolus. (*b*) 46 mm. Hg. and 40 mm. Hg. (2-9, 10)

10. Carbon dioxide has a greater coefficient of diffusion than oxygen. (2-10)

11. (*a*) Arterial blood contains more oxygen than does venous blood.
(*b*) No; the pulmonary artery contains venous blood which is enroute to the lungs.
(2-14)

12. (*a*) Hemoglobin is a complex molecule of rather high molecular weight (64,500 a.m.u.). It contains 4 atoms of iron surrounded by chains of animo acids. (2-15)
(*b*) Hemoglobin oxidizes to the ferric (+3) state and thus becomes oxyhemoglobin. In giving up oxygen, oxyhemoglobin reduces to the ferrous state. (2-17)

13. (*a*) Macrophages engulf large particles such as protozoa as well as small particles like bacteria. Microphages engulf small particles only.
(*b*) Monocytes are macrophages and neutrophils are microphages. (2-20, 21)

14. The lymphocyte is believed to be directly connected with antibody production. (2-24)

15. (*a*) Right ventricle – pulmonary artery – lungs – pulmonary vein – left atrium.
(*b*) Left ventricle – aorta – superior and inferior vena cava – right atrium.
(2-28, 29)

16. There are at least two factors to consider here. First, there may have been a change which was within range of allowable error. For example, if the range of allowable error is ±0.5 g%, the first hemoglobin *could* have actually been 11.5, and the second 10.5. Second, a patient who bleeds severely will show a decrease in blood volume and a decrease in the *total hemoglobin,* but not an immediate decrease in *hemoglobin concentration* until intravenous or tissue fluids have moved into the vascular system to restore the blood volume and dilute the red cells. (2-31-33)

CHAPTER 2

1. (*a*) Venipunctures provide samples of blood adequate for repeating procedures.
   (*b*) The venipuncture is a more sophisticated technique with the inherent disadvantage of scarring the patient's venipuncture site. This makes subsequent venipuncture more difficult.
   (*c*) No.
   (Intro.-2)
2. The lateral surface just beyond the distal joint of a finger in adults and children. (3-3)
3. (*a*) A "T" or criss-cross incision in the heel.
   (*b*) Care must be taken not to cut a tendon.
   (3-3)
4. The needle goes straight in and out without tearing tissue or causing undue pain and discomfort. (3-4)
5. You never draw blood from either the femoral vein, or from the jugular vein. (3-6)
6. In coagulation studies. (3-6)
7. It is a cleansing agent, not a disinfectant or effective antiseptic. (3-8)
8. Release the tourniquet, withdraw the needle, and apply pressure to the venipuncture site. (3-9)
9. Bending the elbow may actually restrict the venous return of blood if the patient's sleeve is restricting. (3-10)
10. (*a*) Heparin.
    (*b*) EDTA.
    (*c*) Cost
    (3-12, 13)
11. (*a*) $\frac{.3}{(101-1)} = \frac{.3}{100} = 1:333$
    (*b*) In cases of high red count, e.g., polycythemia.
    (4-7)
12. (*a*) 200.
    (*b*) 10.
    (*c*) 5.
    (4-10)
13. (*a*) 20.
    (*b*) 10.
    (*c*) 4.
    (4-13)
14. $\frac{9 \times 10 \times 1}{9} = 10$ cells per mm.^{3}. (4-15)
15. The predominant type of cell is an indication of the disease process. For example, infections due to bacteria produce an increase in the neutrophils. (4-17)
16. 15 – 30 minutes, 3 hours, and 6 hours. (4-21)
17. (*a*) Semen diluting fluid ingredients:
    Sodium bicarbonate – 5 gm.
    Formalin – 1 ml.
    Distilled water – 100 ml.
    (*b*) The diluting fluid dissolves the mucus and stops the sperm from moving so that they can be accurately counted.
    (4-22)

18. (a) 127 × 20 × 5 × 10 × 1000 = 127,000,000/ml.
(b) 60 - 150 million/ml.
(4-22)

19. (*a*) Diluted cells are pumped through the counting zone. As the cells pass through a counting area, a photoelectronic impulse is produced and these impulses are counted.
(*b*) Blood cells resist current flow proportional to the number of cells present. A digital counter approximates the number of blood cells which impede current flow.
(4-23)

20. Dilution inaccuracies and false impulses due to interference or contamination. (4-27)

21. (*a*) Repetitive counts at intervals of 5 minutes or less will reveal the point at which WBC's decrease.
(*b*) 15 minutes.
(4-28)

22. Observe the mercury travel time; record the background count; record the control count; flush the orifice. (4-30)

23. Recognize normal cell types. (5-4)

24. Use an ocular micrometer. (5-9)

25. The cytoplasm mass is twice that of the nuclear mass. (5-12)

26. (*a*) If the nucleus is more indented than bean-shaped, the cell is a band.
(*b*) If filaments are present, the cell is a neutrophil.
(5-14)

27. The presence of nucleoli. (5-15)

28. Place the smears in 95 percent methanol for about 30 minutes. (5-21)

29. Prepare fresh stain, buffer, or both. The pH of the stain and/or buffer should be increased. (5-26)

30. 4 to 6. (5-29)

31. (*a*) ±0.5 grams percent.
(*b*) Errors due to incorrect blood volumes and pipetting errors in general.
(6-4)

32. Incorrect pipette calibration; dirty cuvettes or dirty pipettes; instrument errors; incorrect dilutions; unmatched cuvettes (flow-through instruments excepted); faded blank; and deteriorated Drabkin solution. (6-9)

33. The time required for complete reaction is critical. (6-10)

34. (*a*) Methemoglobin, carboxyhemoglobin, and sulfhemoglobin.
(*b*) These forms are not readily converted to oxyhemoglobin.
(6-12)

35. Misidentification; nonrepresentative sample; inadequate sealing of capillary tube; slanting the cell layer after centrifugation; under-centrifugation. (6-14, 15)

36. (1) To determine daily or monthly standard deviation as calculated from a series of duplicate determinations according to the formula

$$\text{Standard deviation} = \sqrt{\frac{\Sigma(a-b)^2}{2n}}$$

Where a = first value of Specimen X
b = second value of Specimen X
$\Sigma(a-b)^2$ = sum of the difference of all duplicates squared
n = number of duplicates.

(2) Standard deviation may also be expressed as a percentage of the mean. (Coefficient of variation.) (7-5, 8)

CHAPTER 3

1. Qualitative variations in red cells include differences in size, shape, internal structure, and type of hemoglobin. (Intro.-4)
2. Erythropoiesis is the production and development of red blood cells. (8-1)
3. The biconcave disk shape facilitates oxygen exchange by increasing the surface area of the red blood cell. (8-2)
4. Prorubricyte. (8-3)
5. The rubriblast. (8-4)
6. The unique (spoke-wheel) chromatin pattern and appearance of polychromatophilia are the most distinguishing characteristics of the rubricyte. (8-6)
7. The metarubricyte. (8-7)
8. A supravital stain is required to observe reticulocytes, but both diffuse basophilia and basophilic stippling are observed in Wright stained blood smears. Reticulocytes are normal cells (0.5 to 1.5%) in adult peripheral blood, while RBCs with diffuse basophilia and basophilic stippling are not. (8-8, 9)
9. This cell is thinner in the center (biconcave) so that less cytoplasm is stained. (8-11)
10. The growth rate is synchronized. (8-12)
11. Asynchronism is erythropoiesis in which the development rate of the nucleus and cytoplasm is *not* synchronized. An example is the megaloblast series of erythrocyte precursors in pernicious anemia. (8-13)
12. Sickle cells, elliptocytes, and target cells. (8-14)
13. Decreased hemoglobin content. (8-16)
14. The terms refer to the presence of residual basophilic substance in the cytoplasm which persists from more immature red cell precursors. (8-10, 17)
15. A spherocyte is spherical in shape and has a smaller diameter and darker color than normal erythrocytes. (8-18)
16. Sickle cells on a differential blood smear might mean either the existence of sickle cell trait or sickle cell disease. (8-21)
17. Cabot rings. (8-24)

18. Nuclear remnants; Howell-Jolly bodies; Cabot rings. (8-23, 24)

19. A siderocyte is an erythrocyte containing iron deposits which may be demonstrated with Prussian blue stain. (8-27)

20. The usual spherocyte is a small round or teardrop-shaped erythrocyte with an even margin. Acanthocytes are spherocytic erythrocytes with long spiny projections from the margins of the cell. (8-18, 28)

21. If the ratio of red cell production is greater than red cell destruction so that total red cell volume is increased, the condition is known as polycythemia. (9-2)

22. Relative polycythemia; secondary polycythemia; polycythemia vera. (9-3-5)

23. An alteration in the quality of RBCs may also indicate anemia. (9-6)

24. (*a*) Normochronic – normal amount.
    (*b*) Hypochromic -- less than normal amount.
    (9-7)

25. *a*. Diet.
    b. Intrinsic.
    c. Vitamin B_{12}.
    d. Folic acid.
    e. Gastric absorption.
    f. Liver storage. (9-8)

26. A hemoglobinopathy is an inherited abnormality of hemoglobin. (9-9)

27. (*a*) Sickle cell anemia.
    (*b*) Thalassemia. (9-9)

28. Free hemoglobin impairs the oxygen-carrying capacity of red blood cells. (9-11)

29. Congenital hemolytic anemia. (9-11)

30. Antibodies weaken the cellular membrane. (9-13)

31. Fragility tests. (9-17)

32. The cells would be smaller than normal size and exhibit an increased central pallor. (8-14, 16; 9-18)

33. The anemia results from incompatible antibodies in the newborn *acquired* from the mother. (9-11-13, 19)

34. Prolonged exposure to any of the following:
    (*a*) Benzene (glue sniffing.)
    (*b*) Arsenic.
    (*c*) Gold salts.
    (*d*) Radioactivity.
    (*e*) Certain antibiotics.
    (9-24)

35. Red cells that contain hemoglobin "S" will form sickle shapes when placed under lowered oxygen tension. (10-2)

36. The test should be reported *only* as positive or negative. (10-3)

37. The ESR is used in a general sense to detect, confirm, and/or follow the course of a disease process. (10-4)

38. The mass of a falling particle in solution is directly proportional to its rate of fall. An increase in mass increases the rate of fall. (10-5)

39. (1) Roulleaux formation.
    (2) Autoagglutination.
    (3) Severe macrocytosis.
    (10-6)

40. *a.* Length and bore size of tube.
    b. Level of tube support rack.
    c. Vibrations.
    d. Anticoagulant.
    e. Specimen age.
    f. Temperature.
    (10-10-12)

41. Plasma protein. (10-13)

42. *a.* Mean corpuscular volume (MCV).
    b. Mean corpuscular hemoglobin (MCH).
    c. Mean corpuscular hemoglobin concentration (MCHC).
    (10-14)

43. *a.* Red blood cell count.
    b. Hematocrit.
    c. Hemoglobin.
    (10-15)

44. The threshold cannot be set for a normocytic RBC population. (10-17)

45. Osmotic pressure of the fluid. (10-18)

46. Crenation occurs. (10-19)

47. The osmotic pressures are balanced between the cell and saline. (10-20)

48. The blood specimen may be collected aseptically in a sterile container with glass heads and incubated 24 hours at 37° C. before running the test. (10-22)

CHAPTER 4

1. (1) Cells decrease in size as they mature.
   (2) Nuclear chromatin becomes more clumped (compact).
   (3) Cytoplasm staining reaction changes.
   (4) Younger cells are more basophilic.
   (11-1)

2. Azurophilic or nonspecific granules. (11-2, 3)

3. *a.* Myeloblast.
   b. Promyelocyte.
   c. Myelocyte.
   d. Metamyelocyte.
   e. Band.
   f. Segment.
   (11-3)

4. *a.* The unique, delicate, hemogeneously diffused chromatin net, with 2-5 distinct nucleoli in the nucleus and the absence of cytoplasmic granules are the most differentiating morphological characteristics of the myeloblast.

   b. Promyelocyte. The appearance of a few azurophilic granules in the cytoplasm is immediately apparent in differentiating this cell. You will note that neither the myeloblast, which precedes this cell, nor the myelocyte which follows, has cytoplasmic, azurophilic granules alone. The indistinct nucleoli and more coarse chromatin may confirm the identification, but are more relative and subjective morphological distinctions.

   c. Myelocyte. The persistence of the nuclear shape – round or oval as in the precursors – and appearance of definitive granules differentiate this cell. Azurophilic granules may or may not be present, so they are not characteristic features.

   d. Metamyelocyte. Although these cells have subtle morphological differences in N:C ratio, granulation and chromatin net, the differentiating characteristic common to all three is nuclear shape. All three cell types have definitive granules. The metamyelocyte has an indented or kidney shaped nucleus. When the nucleus is more indented than kidney shaped but not filamented, the cell is classed as a band. (11-4-10)

5. The percentage of these cells is normally low and their differentiation serves no useful clinical purpose. (11-13, 15)

6. The large size (40-100 microns) and platelets either in or fragmented from the cytoplasm are distinguishing features of the megakaryocyte. (11-20)

7. The lymphatic system. (11-21)

8. *a.* Cell size. 6-20 microns in diameter or from about the size of an RBC to twice the size of an RBC.
   b. Quantity of cytoplasm. From scant to moderate amount.
   c. Cytoplasm histochemistry. Light to moderately basophilic. Cytoplasm staining. From light to moderate blue.
   d. Cytoplasmic granules. Reddish violet or azurophilic, peroxidase negative.
   e. Nuclear shape. Round, oval, or slightly indented.
   f. Chromatin. Clumped, with dark staining bluish purple aggregates separated by indistinct areas of parachromatin.
   (11-25)

9. A monoblast. (11-27)

10. The very fine, diffuse chromatin strands with abundant parachromatin. (11-30)

11. Spindle forms; smudge or basket cells. (12-1)

12. Leukocytosis and leukopenia. (12-2)

13. There is no correlation. (12-12)

14. Vacuolated cytoplasm, deeply basophilic cytoplasm, and nucleoli observed singly or in any combination. (12-13)

15. The leukocyte alkaline phosphatase test, which is low in granulocytic leukemias. (12-17)

16. Acute lymphocytic leukemia. (12-19)

17. Thin, eosinophilic, rod-shaped structures observed in the cytoplasm of some granulocytes in acute granulocytic leukemia. (12-19)

18. A true L.E. cell is a granulocyte with a hemogeneous, cytoplasmic inclusion which has *no* chromatin pattern. A tart cell is a granulocyte containing a phagocytized nucleus with a recognizable chromatin pattern. (12-21)

CHAPTER 5

1. (*a*) *Hemostasis* is a complex process in which several factors work together or in sequence to stop the flow of blood from an injured blood vessel.
   (*b*) The *extravascular mechanism* involves the surrounding tissue such as skin, muscle, and subcutaneous tissue. These tissues help stop the flow of blod by contracting or by virtue of their weight and thickness.
   (*c*) The *vascular mechanism* involves the veins, arteries, and capillaries themselves. They aid in stopping the flow of blood by contracting and retracting to close off the vessel.
   (*d*) The *intravascular mechanism* involves coagulation of the blood itself. This is a highly complicated mechanism that requires several stages for completion. These stages are the generation of thromboplastin, the generation of thrombin, and the production of fibrin.
   (13-2)

2. (1) The formation of thromboplastin.
   (2) The conversion of prothrombin to thrombin.
   (3) The formation of an insoluble fibrin clot through the interaction of fibrinogen and thrombin.
   (13-5)

3. (*a*) The factors responsible for coagulation are found in the plasma.
   (*b*) Tissue thromboplastin.
   (13-6, 7)

4. The partial thromboplastin time indicates an abnormality in one or more of the clotting factors except Factor VII or platelet deficiencies. (13-14)

5. (*a*) Stage II.
   (*b*) Factors II, V, VII, and X.
   (13-18)

6. (*a*) Hemophilia A involves factor VIII.
   Hemophilia B involves factor IX. (13-21)
   (*b*) Hemophilia is inherited as a trait carried on the X-chromosome. Since males have only one X-chromosome, they would be more likely to show the expressed condition. (13-21)

7. The capillary fragility (tourniquet) test. (14-2-3)

8. No; although widely used, the bleeding time test incorporates too many variables which are not easily controlled. (14-4)

9. Platelet deficiency. (14-10)

10. Debris can be distinguished on the basis of refractility, angular shape, and absence of a pink-purple sheen. (14-15, 16)

11. Settling in the pipette with resultant uneven distribution of platelets. (14-17)

12. (1) Perform the test soon after the blood has been collected.
    (2) Maintain careful temperature control.
    (3) Exercise accurate timing (an automatic timer is preferred.)
    (4) Use a high quality freshly prepared thromboplastin extract.
    (5) Run a suitable control.
    (14-24)

13. (*a*) Stage 1.
    (*b*) The prothrombin time.
    (14-25, 26)

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14. (*a*) Deficiencies in prothrombin, Factor V, and any of the factors in the intrinsic system.
(*b*) The prothrombin time test.
(14-29)

15. A specific inhibitor against thromboplastin formation is possible. (14-31)

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STOP– 1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER. 2. USE NUMBER 1 PENCIL.

90413 01 21

VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp # 1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'T

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a # 1 black lead pencil.

Note: The 3-digit number in parenthesis immediately following each item number in this Volume Review Exercise represents a Guide Number in the Study Reference Guide which in turn indicates the area of the text where the answer to that item can be found. For proper use of these Guide Numbers in assisting you with your Volume Review Exercise, read carefully the instructions in the heading of the Study Reference Guide.

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Multiple Choice

Note: The first three items in this exercise are based on instructions that were included with your course materials. The correctness or incorrectness of your answers to these items will be reflected in your total score. There are no Study Reference Guide subject-area numbers for these first three items.

1. The form number of this VRE must match

   a. my course number.
   b. the number of the Shipping List.
   c. the form number on the answer sheet.
   d. my course volume number.

2. So that the electronic scanner can properly score my answer sheet, I must mark my answers with a

   a. pen with blue ink.
   b. ball point or liquid-lead pen.
   c. number 1 black lead pencil.
   d. pen with black ink.

3. If I tape, staple or mutilate my answer sheet; or if I do not cleanly erase when I make changes on the sheet; or if I write over the numbers and symbols along the top margin of the sheet.

   a. I will receive a new answer sheet.
   b. my answer sheet will be unscored or scored incorrectly.
   c. I will be required to retake the VRE.
   d. my answer sheet will be hand-graded.

Chapter 1

4. (100) Under normal conditions, an adult's red blood cells are produced in

   a. the spleen.
   b. the red marrow of the spongy bones.
   c. both the spleen and the red marrow.
   d. the yellow marrow of the long bones.

5. (100) The liver begins to produce blood cells

   a. in the first month of fetal life.
   b. 6 weeks after birth.
   c. at birth.
   d. the second month of fetal life.

6. (100) The level of fibrinogen in normal human plasma is from

   a. 0.15 to 0.30 mg. percent.
   b. 0.15 to 0.30 g. percent.
   c. 0.30 to 0.55 mg. percent.
   d. 0.30 to 0.55 g. percent.

7. (101) When oxygen reaches a partial pressure of 100 mm. Hg. in the capillaries during external respiration, the saturation of hemoglobin reaches

   a. 20.
   b. 50.
   c. 98.
   d. 100.

8. (100) Leukocyte production is believed to begin

   a. in the third month of fetal like in the liver.
   b. in the seventh month of fetal life in the spleen.
   c. at birth.
   d. in the early stages of fetal blood formation.

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9. (102) The cell most closely associated with allergic reactions is the

a. basophil.
b. monocyte.
c. lymphocyte.
d. eosinophil.

10. (101) The exchange of oxygen and carbon dioxide between the alveoli and the blood stream is best described as

a. breathing.
b. internal respiration.
c. external respiration.
d. expiration.

11. (102) Which of the following sequences is correct for blood flow through the pulmonary circulatory system?

a. Superior and inferior vena cava, right atrium, aorta, and left atrium.
b. Left ventricle, aorta, vena cava, and right atrium.
c. Pulmonary artery, lungs, aorta, and right atrium.
d. Right ventricle, pulmonary artery, lungs, pulmonary vein, and left atrium.

12. (101) The exchange of oxygen between the blood and other tissue cells is called

a. tissue exchange.
b. osmosis.
c. external respiration.
d. internal respiration.

13. (101) The so-called normal form of hemoglobin is designated by the letter

a. A.
b. F.
c. C.
d. S.

14. (101) The maintenance of a biologically balanced internal environment of the body is termed

a. hemostasis.
b. homotonicity.
c. homozygosity.
d. homeostasis.

15. (100) Another name for *thrombocytes* is

a. megalocytes.
b. platelets.
c. megakaryocites.
d. lymphocytes.

16. (102) Antibodies are thought to be produced by the

a. lymphocyte.
b. monocyte.
c. spleen.
d. megakaryocyte.

17. (101) The effective partial pressure differential of CO_2 in mm. Hg. between the tissue cell and the capillary reaches

a. 46.
b. 40.
c. 6.
d. 4.

18. (101) If air in the alveolus at sea level contains water vapor at a pressure of 47 mm. Hg., the resultant effective gas pressure in the alveolus

a. is 713.
b. is 47.
c. is 807.
d. cannot be determined from data given.

19. (102) Which of the following sequences is correct for blood flow through the systemic circulatory system?

a. Right ventricle, pulmonary artery, and lungs.
b. Aorta, left ventricle, and vena cava.
c. Pulmonary vein, left atrium, and right ventricle.
d. Left ventricle, aorta, vena cava, and right atrium.

Chapter 2

20. (105) Dyes used to stain living cells are termed

a. polychromatic.
b. supra-vital.
c. panoptic.
d. viable.

21. (106) Errors in hemoglobinometry usually occur

a. in pipetting blood.
b. in pipetting diluent.
c. because of variable standards.
d. in spectrophotometric variations.

22. (107) Coefficient of variation is expressed by the formula

a. mean X standard deviation.
b. $100 \times \frac{\text{standard deviation}}{\text{mean}}$
c. 100 X standard deviation.
d. $\sqrt{\frac{\Sigma (a-b)^2}{2N}}$

23. (104) Which of the following is *not* usually a source of error in electronic cell counting?

a. Wornout arcing brushes.
b. Dilution errors.
c. Cellular debris.
d. Erroneous impulses.

24. (103) Isopropyl alcohol (70 percent) is best described as

a. a disinfectant.
b. an antiseptic.
c. a sterlizing agent.
d. a cleansing agent.

25. (104) In an adult female, the normal range of erythrocytes in million per cmm. is from

a. 2.6 to 3.8.
b. 3.8 to 4.6.
c. 4.2 to 5.4.
d. 4.6 to 6.2.

26. (105) The normal average number of platelets on a differential blood smear per oil immersion field is from

a. 0 to 2.
b. 4 to 6.
c. 8 to 10.
d. 12 to 14.

27. (105) A cell is classified as a segmented neutrophil rather than a band cell if

a. the nucleus is indented more than halfway.
b. filaments are visible.
c. neutrophilic granules predominate.
d. the filaments are not visible.

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28. (104) Yellow coloration of CSF is commonly referred to as

a. spinalgia.
b. xerasia.
c. xanthochromia.
d. polychromasia.

29. (103) Blood smears for differentials are best prepared from blood containing

a. EDTA.
b. heparin.
c. double oxalate.
d. no anticoagulant.

30. (105) All differential blood smears should be kept in the laboratory for at least

a. 1 week.
b. 6 weeks.
c. 3 months.
d. 6 months.

31. (106) Drabkin solution should not be discarded into a sink immediately preceded or followed by

a. strong alkalies.
b. acids.
c. cyanide salts.
d. hot water.

32. (104) Electronic cell counters employing the *principle of resistance* measure

a. impedence.
b. cell size.
c. photoelectric impulses.
d. all of the above.

33. (104) If blood is filled to the 1 mark in a WBC pipette and diluted to the 11 mark, the resultant dilution is from 1 to

a. 10.
b. 11.
c. 100.
d. 111.

34. (104) The usual normal range for a sperm count in number per ml. is from

a. 10,000 to 100,000.
b. 100,000 to 250,000.
c. 600,000 to 1 million.
d. 60 million to 300 million.

35. (107) The mean standard deviation is most meaningful if calculated

a. for each test.
b. daily.
c. weekly.
d. monthly.

36. (105) If in doubt as to whether a cell should be classified as a metamyelocyte or a band, you would probably

a. omit the cell from your count.
b. classify it as a metamyelocyte.
c. classify it as a band.
d. note your indecision in the "Remarks" section of SF 514.

37. (105) Every 90470 technician is expected to

a. recognize normal blood cell characteristics.
b. identify all abnormal blood cells.
c. classify lymphocytes.
d. do all of the above.

38. (106) The least significant error in the microhematocrit determination is

a. undercentrifugation.
b. overcentrifugation.
c. presence of tissue fluid from a capillary puncture.
d. failure to mix the blood from a test tube.

39. (104) Which of the following statements concerning electronic cell counts is true?

a. One control may be used to control one entire group of procedures.
b. Most laboratories allow plus or minus 5-percent variation for different dilutions of the same sample.
c. Bubbles may be counted as cells.
d. All of the above statements are true.

40. (107) What would you expect the coefficient of variation for manual counts to be in comparison to that of electronic cell counts?

a. Greater.
b. Less.
c. Equal
d. Insignificantly different.

41. (103) The primary reason for stoppering blood collection tubes is to prevent

a. metabolism of the cells.
b. evaporation.
c. the spread of disease.
d. contamination.

42. (104) When semen is received in the laboratory, it should be

a. stored at 37° C. until it liquefies.
b. allowed to remain at room temperature until liquefaction.
c. examined in the gelatinous state immediately.
d. refrigerated until examined.

43. (103) The principal advantage of venous blood over capillary blood for routine hematology is that

a. a larger volume of sample is obtained.
b. venous blood contains less oxygen.
c. the number of cells per cu. mm. in venous blood is more representative of the circulatory system.
d. the veins are larger and easier to enter.

Chapter 3

44. (110) A clinical test in hematology which is used in a general sense to detect, confirm, and/or follow the course of a disease process is the

a. sickle cell prep.
b. RBC fragility.
c. sedimentation rate (ESR).
d. MCH.

45. (108) Which of the following is a quantitative variation of erythrocytes?

a. Poikilocytosis.
b. Packed cell volume.
c. Low red cell count.
d. Target cell.

46. (108) In normal erythropoiesis, a cell precursor to the erythrocyte which cannot be observed on Wright stained smears is

a. a polychromatophilic RBC.
b. a reticulocyte.
c. an orthochromatic RBC.
d. a normocyte.

47. (108) Small, round, refractile inclusions inside the erythrocyte, visible only in unfixed smears, are

a. Howell-Jolly bodies.
b. Heinz-Ehrlich bodies.
c. siderocytes.
d. acanthocytes.

48. (108) Abnormal red cells which have a half-moon or sickle-shaped appearance

a. appear under conditions of reduced oxygen tension.
b. are positive evidence of anemia.
c. are found in about 10 percent of all whites.
d. occur only in about 1 percent of Negroes.

49. (109) With hemolysis, the oxygen-carrying capacity of RBC's is impared by

a. ionic iron.
b. cellular stroma.
c. cellular membrane.
d. free hemoglobin.

50. (110) Since the mean corpuscular volume (MCV) is a mean value, it may be normal even with

a. microcytosis.
b. macrocytosis.
c. anisocytosis.
d. all of the above.

51. (109) Which of the following may cause aplastic anemia?

a. Chronic hemorrhage.
b. Glue sniffing.
c. Malaria.
d. Congenital defect.

52. (108) Which of the following is a type of cell normally found in adult peripheral blood?

a. Reticulocyte.
b. Polychromatophilic RBC.
c. Diffusely basophilic RBC.
d. Metarubricyte.

53. (110) Technically, the anticoagulants of choice for the erythrocyte sedimentation rate are

a. EDTA and double oxalate.
b. heparin and EDTA.
c. heparin and double oxalate.
d. double oxalate and sodium metabisulfate.

54. (108) A megalocyte is normally

a. polychromatophilic.
b. a small cell.
c. a large cell.
d. basophilic.

55. (110) The effect of anemia on the erythrocyte sedimentation rate is described as

a. linear.
b. subject to correction.
c. inconsequential.
d. nonlinear.

56. (108) Nucleoli can only be found in which of the following cells of the rubricytic series?

a. Rubricyte.
b. Proubricyte, rubriblast.
c. Metarubricyte, rubricyte.
d. Rubriblast.

57. (110) The degree of red cell fragility is directly related to the

a. osmotic pressure.
b. atmospheric pressure.
c. cell membrane.
d. cell stroma.

58. (108) An iron-porphyrin ring compound that readily takes up and liberates oxygen is

a. sodium metabisulfite.
b. hemoglobin.
c. new methylene blue.
d. stercobilinogen.

59. (109) A red blood cell which contains 35 percent hemoglobin would be described as

a. polychromatophilic.
b. hyperchromic.
c. normochromic.
d. hypochromic.

60. (110) Red cell fragility is increased in

a. sickle cell anemia.
b. congential spherocytic anemia.
c. Mediterranean anemia.
d. thalassemia.

Chapter 4

61. (112) The classic Downey cells in infectious mononucleosis are excellent examples of

a. hyperplasia.
b. polycythemia vera.
c. erythrocytes.
d. atypical lymphocytes.

62. (111) The importance of megakaryocytes may lie in coagulation problems involving

a. thrombocytes.
b. megalocytes.
c. normocytes.
d. myelocytes.

63. (112) In which of the following leukemias are leukocytes low in the alkaline phosphatase test?

a. Acute myelocytic.
b. Acute lymphocytic.
c. Chronic lymphocytic.
d. Granulocytic.

64. (111) The appearance of only a few large, nonspecific azurophilic, cytoplasmic granules in a cell of the granulocytic series definitely identifies a

a. myelocyte.
b. myeloblast.
c. promyelocyte.
d. metamyelocyte.

65. (111) Eosinophils found in normal peripheral blood smears are comparable in size to

a. meutrophilic segmented cells.
b. basophils.
c. neutrophils.
d. myelocytes.

66. (112) In acute granulocytic leukemia, thin, eosinophilic, rod shaped structures in the cytoplasm are called

a. toxic granulation.
b. Auer rods.
c. definitive granules.
d. reticulums.

67. (111) What morphological characteristic in the lymphocyte and metamyelocyte clearly differentiates them from the monocyte?

a. Clumped chromatin.
b. Nucleoli.
c. Specific granules.
d. Perinuclear zone.

68. (112) An absloute lymphocytosis would suggest that in the differential, a technician should observe closely for

a. vacuolated cytoplasm.
b. deeply basophilic cytoplasm.
c. the presence of nucleoli.
d. all of the above.

69. (111) A general rule in hemopoiesis is that, as the cells mature, the nuclear chromatin becomes more

a. clumped.
b. basophilic.
c. reticulated.
d. delicate.

70. (112) The Downey classification of atypical lymphocytes in infectious mononucleosis is

a. essential.
b. prognostic.
c. of historical interest.
d. diagnostic.

71. (111) The differentiation of cells more mature than the myelocyte is based almost exclusively on

a. cytoplasmic granules.
b. nuclear configuration.
c. nuclear chromatin.
d. cell size.

72. (112) A polymorphonuclear leukocyte containing engulfed nuclear material with a distinct chromatin net is

a. a pus cell.
b. a rosette.
c. an L.E. cell.
d. a tart cell.

73. (112) A shift to the left in differential counting indicates neutrophilic

a. hyperlobulation.
b. degeneration.
c. immaturity.
d. maturation.

74. (112) If the total leukocyte count is below 4,000/cmm., the term used is

a. leukopenia.
b. leukocytosis.
c. leukemia.
d. neutropenia.

Chapter 5

75. (113) The conversion reaction of fibrinogen to fibrin is best described as

a. an endothermic reaction.
b. an autolytic reaction.
c. an enzymatic reaction.
d. a catalytic reaction.

76. (115) The so-called one-stage prothrombin time measures

a. stage I defects only.
b. stage II defects only.
c. stage III defects only.
d. defects in stages II and III.

77. (113) The thrombin time test measures

a. a deficiency of fibrinogen.
b. the function of proaccelerin.
c. the Hagemen factor deficiency.
d. prothrombin consumption.

78. (113) The prothrombin consumption test theoretically measures

a. converted prothrombin.
b. unconverted prothrombin.
c. specific clotting defects.
d. coumarin.

79. (114) The principal disadvantage of the finger puncture bleeding time test is that it

a. lacks standardization.
b. gives pain to the patient.
c. is seldom abnormal.
d. does not measure the intravascular mechanism.

80. (113) The second stage of the clotting mechanism involves the

a. dissolution of fibrinogen.
b. formation of thromboplastin.
c. conversion of prothrombin to thrombin.
d. interaction of fibrinogen and thrombin.

81. (114) A prolonged clotting time would *not* reveal significant

a. fibrinolysin deficiency.
b. circulating anticoagulants.
c. platelet deficiencies.
d. defects in stage I.

82. (115) Reese-Ecker solution for the platelet count contains

a. methylene blue.
b. fluorescein.
c. brilliant cresyl blue.
d. crystal violet.

83. (115) In order for the prothrombin consumption test to be valid,

a. Factor VII must be added.
b. the prothrombin time must be normal.
c. platelets must be adequate.
d. all of the above conditions must be fulfilled.

84. (113) If a hemophilic man is married to a normal female, one would expect their

a. sons to be hemophilic.
b. daughters to be hemophilic.
c. sons to be carriers.
d. sons to be normal.

85. (115) In performing a platelet count, the most likely error occurs through

a. overshaking the pipette.
b. settling in the pipette.
c. settling in the chamber.
d. uneven distribution in the chamber.

86. (115) Failure to obtain significant correction when normal reagents are substituted in the thromboplastin generation test suggests the

a. absence of sufficient calcium.
b. presence of a thromboplastin precursor inhibitor.
c. deficiency of Factor X (Stuart).
d. test is invalid.

87. (113) Which of the following factors is used to confirm the diagnosis of hemophilia?

a. Factor I.
b. Factor II.
c. Factor VII.
d. Factor VIII.

88. (113) Incubation of a normal clot with the patient's plasma would reveal a defect in

a. fibrinolysin.
b. prothrombin.
c. fibrinogen.
d. fibrin.

89. (114) Clot retraction may appear increased if the patient has a

a. decrease in plasma volume.
b. high fibrinogen level.
c. low fibrinogen level.
d. decrease in red cell mass.

90. (115) Barium adsorbed plasma used in the prothrombin consumption test must contain all of the following *except*

a. fibrinogen.
b. Factor V.
c. Factor VII (stable factor).
d. both Factor V and fibrinogen.

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CDC 90413

MEDICAL LABORATORY TECHNICIAN-HEMATOLOGY, SEROLOGY, BLOOD BANKING, AND IMMUNOHEMATOLOGY

(AFSC 90470)

Volume 2

Laboratory Procedures in Blood Banking and Immunohematology

10-4

Extension Course Institute

Air University

109

Preface

THIS SECOND volume of Course 90413 is concerned with blood banking and immunohematology. Chapter 1 discusses antigen and antibody reactions in their relationship to blood banking. Major blood group systems are discussed in Chapter 2. The special precautions and procedures used in preparing blood for transfusions is presented in Chapter 3. The last chapter of the volume is devoted to the operation of a blood donor center.

Some of the material has been illustrated on foldouts. When the text discusses this material, open the foldout at the back of the text and refer to it as you study.

A glossary of technical terms is included in Volume 3.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to SHCS (MST/114), Sheppard AFB TX 76311.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Study Reference Guides, Chapter Review Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFB AL 36118, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 18 hours (6 points).

Material in this volume is technically accurate, adequate, and current as of May 1973.

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LIST OF CHANGES

COURSE NO. 90413	CAREER FIELDS, POLICIES, PROCEDURES AND EQUIPMENT CHANGE. ALSO ERRORS OCCASIONALLY GET INTO PRINT. THE FOLLOWING ITEMS UPDATE AND CORRECT YOUR COURSE MATERIALS. PLEASE MAKE THE INDICATED CHANGES.
EFFECTIVE DATE OF SHIPPING LIST 24 Oct 75	

1. CHANGES FOR THE TEXT: VOLUME 2

a. Page 1, para 1-4, line 2: Change "48" to "46."

b. Page 24, para 6-10, line 12: Change "while" to "whole."

2. CHANGES FOR THE VOLUME WORKBOOK: VOLUME 1

a. Page 6, Chapter Review Exercises, question 14: Change "or" to "of."

b. Page 12, Chapter Review Exercises, question 14: Insert "are" between "How" and "the."

c. Page 29, Answer For Chapter Review Exercises, answer 8, choice a: Add a colon after "size." Choice b: Add a colon after "cytoplasm" and delete "From." Choice c: Add a colon after "histochemistry" and after "staining." Choice d: Add a colon after "granules." Choice e: Add a colon after "shape." Choice f: Add a colon after "Chromatin."

d. Page 29, Answers For Chapter Review Exercises, answer 18, line 1: Change "hemogeneous" to "homogeneous."

3. CHANGE FOR THE VOLUME WORKBOOK: VOLUME 2

Question 59 is no longer scored and need not be answered.

4. CHANGE FOR THE VOLUME WORKBOOK: VOLUME 3

The following questions are no longer scored and need not be answered: 29 and 78.

NOTE: Change the currency date on all volumes to "December 1973."

CHAPTER 1

Immunohematology

BLOOD HAS FASCINATED men since the first caveman noted that loss of blood meant the end of life. Early attempts to restore blood to the body by replacing it with blood from animals or other human beings most often resulted in death. Medical practice of centuries past often called for bleeding the patient as a means of therapy. It was not until the end of the 19th century that certain mysteries of life were explained through the sciences of genetics and immunology, which emerged to form the basis for immunohematology.

2. Immunohematology is the study of the immune response, with particular reference to blood. Through the science of immunohematology we have learned to administer blood with the reasonable assurance that it will support life rather than end it. We have also learned much about blood disorders, particularly hemolytic disease due to antigen-antibody interaction.

1. Genetics and Immunology

1-1. The foundation for the study of immunohematology is genetics, the study of heredity and inheritance. Human red blood cells are produced under the control of genes. All physical and chemical structures of the body, including characteristics of the red blood cells, are determined by gene inheritance. Blood banking is a practical application of genetic principles. As you read this chapter and the remainder of the volume, certain terms may be unfamiliar to you. For this reason, a glossary is provided at the end of Volume 3.

1-2. **Genetics.** When we type blood with antisera, we are identifying a phenotype. A phenotype is the physical expression of a trait. Blue eyes, blonde hair, and group A blood are all examples of phenotypes. Agenotype is an expression of the nature, number, and arrangement of genes, which are, of course, the biochemical entities that determine the phenotype. For example, group A blood, which is a phenotype, may be determined by the genotype AO or AA. (This will be explained further in paragraph 1-6.) In the clinical laboratory there is no practical way to observe the genes themselves, and we must therefore be content with observing the related effect of gene action. Antiserum reacts with a protein (antigen) on the red blood cell. The presence of the antigen is under direct genetic control, but we do not type for the gene with antiserum. We are testing for evidence of the presence of one or more genes.

1-3. Various genes control the nature of antigens, which occur on a person's red blood cells. The offspring develops red blood cell antigens according to genes inherited from his parents. Let us briefly review the theory of gene inheritance as it applies to the subject at hand.

1-4. A body cell that is not a sex (germ) cell has 48 chromosomes. This includes two sex chromosomes. In females the sex chromosomes, or X chromosomes as they are called, are equal in size. Males have an X chromosome and a smaller Y chromosome. Some blood group genes are carried on the X chromosome. These are said to be sex linked. In this case, only the females have two genes for the trait. Other genes of interest to the blood bank technician also show sex linkage, including the gene for hemophilia, which is carried on the X chromosome. A male manifests hemophilia if he has one gene for the condition. Two genes are required to produce the disease in the female. Such a genetic condition is usually fatal to the ovum. Except for certain sex-linked traits, a person has two or more genes for each antigen characteristic of his blood cells.

1-5. Linkage is not limited to the sex chromosomes. Two genes close to each other at a locus before meiosis (reduction division) tend to be inherited together. This is of practical value because the presence of a trait, known to be linked to another trait, is evidence that a gene is present for the other linked trait as well. This explains why a correlation exists between blood groups and certain hereditary abnormalities. For example, the chance that a person of blood group O will develop a duodenal ulcer is nearly 1.5 times as great as the probability of the same disorder in someone of the other blood groups.[1] (The superscript after each source reference refers to a footnote at the end of the chapter.) This is because the secretion of gastric juice is controlled by a gene in close proximity to the O gene. A similar

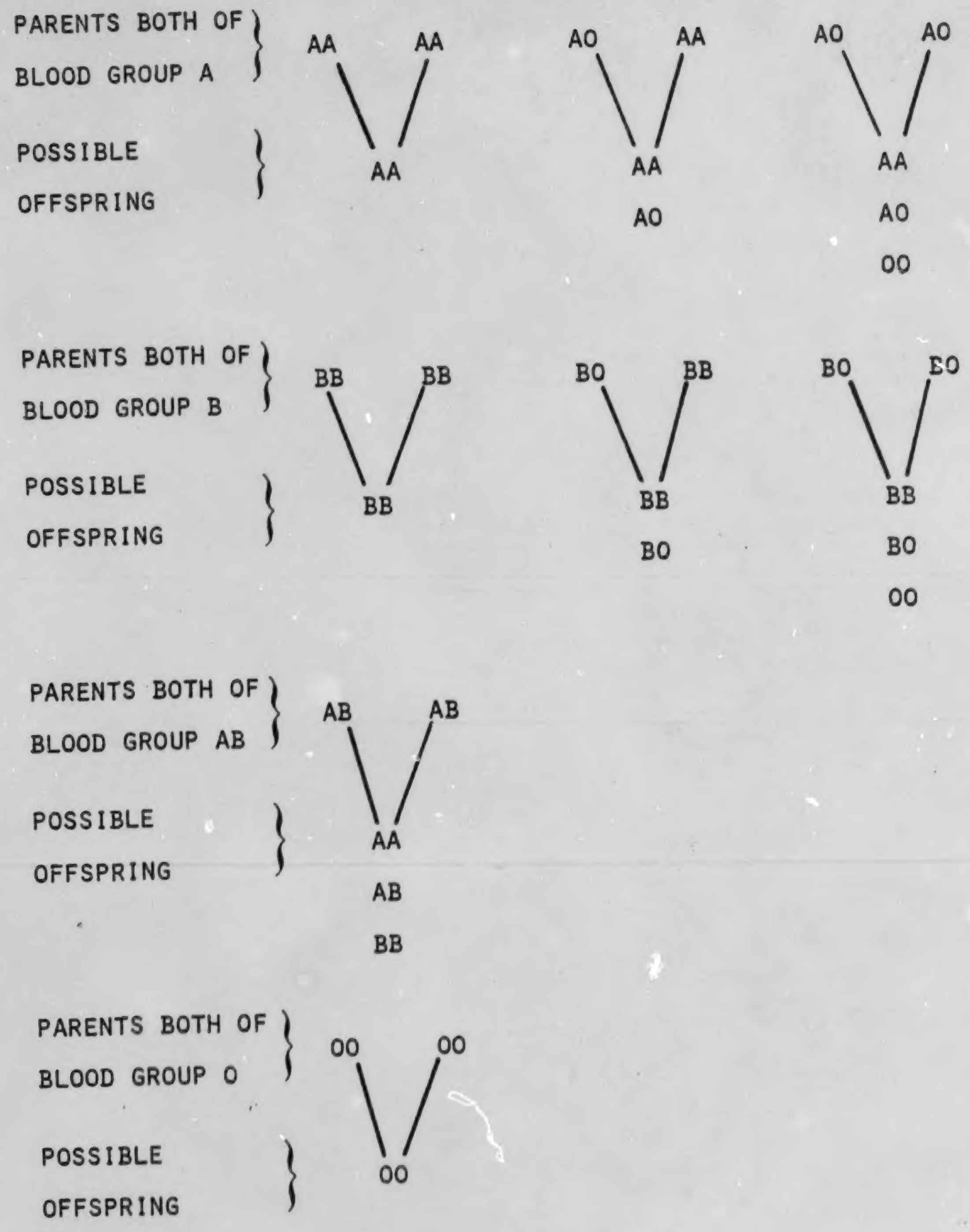

PARENTS BOTH OF BLOOD GROUP A
AA
AA
AO
AA
AO
AO
POSSIBLE OFFSPRING
AA
AA
AO
AA
AO
OO
PARENTS BOTH OF BLOOD GROUP B
BB
BB
BO
BB
BO
BO
POSSIBLE OFFSPRING
BB
BB
BO
BB
BO
OO
PARENTS BOTH OF BLOOD GROUP AB
AB
AB
POSSIBLE OFFSPRING
AA
AB
BB
PARENTS BOTH OF BLOOD GROUP O
OO
OO
POSSIBLE OFFSPRING
OO

Figure 1. Inheritance of ABO blood groups.

comparison can be made with some other disease processes.

1-6. If a person is of blood group A, he has either one or two genes for this trait. The group A individual may be AA or AO (genotype) and still be of blood group A (phenotype). If both genes are alike, e.g., AA, the person is homozygous. If the genes are not alike, e.g., AO, the individual is heterozygous. When this person produces offspring, he passes on only one of the two genes. The same is true of the other parent. The offspring becomes the recipient of two genes, and his blood group depends upon the genes he inherits. Figure 1 illustrates the possibilities with regard to the ABO systems for parents who are both of the same blood group. Of course, parents may be of different blood groups, but the rules of inheritance are the same. Following the reasoning in figure 1, can you predict the possible blood group(s) of offspring from parents in different blood groups? Try writing out some of the possibilities.

1-7. Each gene of a particular pair or group is an allele to the other(s). For instance, in the genotype AO, A and O are alleles to each other. Alleles occur at corresponding positions or loci on homologous (matching) chromosomes. In basic genetics, the concept of dominance and recessiveness is well known. This means that one gene may be dominant over an allele in the effect that is produced. For example, if the gene for dark eyes (B) is dominant over the gene for blue eyes (b), a person who possesses both genes (Bb) will have dark eyes rather than blue eyes. For all practical purposes, genes that control blood groups are neither dominant nor recessive to each other. While it is true that a person who possesses an O gene in addition to either A or B will be grouped as A or B on the basis of cell antigens, the influence of the O gene is there. It simply cannot be detected with available typing sera. A single locus on a chromosome contains an A, B, or O gene, but no more than one of them.

1-8. The Rh system follows the same pattern of genetic inheritance. There are six genes in the Rh system (C, c, D, d, E, e,). The Rh genes from each parent remain together on the same chromosome during fertilization. Thus, the Rh genes on a given chromosome in the offspring represent the same arrangement as those inherited from one of the parents. For example, if the Rh gene grouping on a chromosome of the mother is CDe and the group of Rh genes on a chromosome in the sperm is cde, the offspring is CDe/cde, not some other combination thereof. What is perhaps more complicating in the Rh system is that several genes are involved, rather than the single "Rh factor," as originally proposed. If you consider the D factor, the genes are D (Rh_0) and d (Hr_0). Unfortunately, we do not have anti-d typing serum, and there is consequently some question of gene action in this case. We will discuss the Rh system further in Chapter 2 of this volume. At this point you should concentrate on the theory of inheritance and its application to blood groups.

TABLE 1
PROBABLE OCCURRENCE OF THE COMMON D POSITIVE GENOTYPES

Antigen Present						*Approximate Percent of Population*
D	C	E	c	e		
+	+	-	+	+	DCe/dce	33.0
					DCe/Dce	2.0
+	+	-	-	+	DCe/DCe	18.0
					DCe/dCe	0.8
+	+	+	+	+	DCe/DcE	12.0
					DCe/dcE	1.5
					DcE/dCe	1.5
+	-	+	+	+	DcE/dce	11.0
					DcE/Dce	1.0
+	-	+	+	-	DcE/DcE	2.0
					DcE/dcE	0.3
+	-	-	+	+	Dce/dce	2.0
					Dce/Dce	0.1

1-9. Determination of genetic patterns—i.e., genotype—is of value in prenatal studies, medicolegal cases, and in locating blood for sensitized patients. In prenatal studies, the possibility of maternal isoimmunization to Rh antigens of the infant requires detailed phenotyping of the mother. The phenotype is then used to derive the probable genotype. It is also desirable to type the father of the child in order to determine which antigens may be inherited by the infant. At the present, there is a problem in predicting genetic inheritance based on typing the parents. For example, we have no certain way of knowing whether a parent is homozygous for D (i.e., DD) or whether he is heterozygous (Dd), unless we have access to genealogical studies of the same family. However, we can type for C, c, E, and e, as well as D, in terms of their red blood cell antigens. Once we have determined which genes are present, it is a matter of probability whether the parent is homozygous or heterozygous. Table 1 gives the probability of common Rh positive genotypes. The subject of Rh isoimmunization is further explained in Chapter 2.

1-10. In medicolegal cases it is often possible to rule out paternity by applying the hereditary (Mendelian) law. It can sometimes be stated on the basis of genotype that a particular individual could *not* be the parent of a child. Can you imagine how a

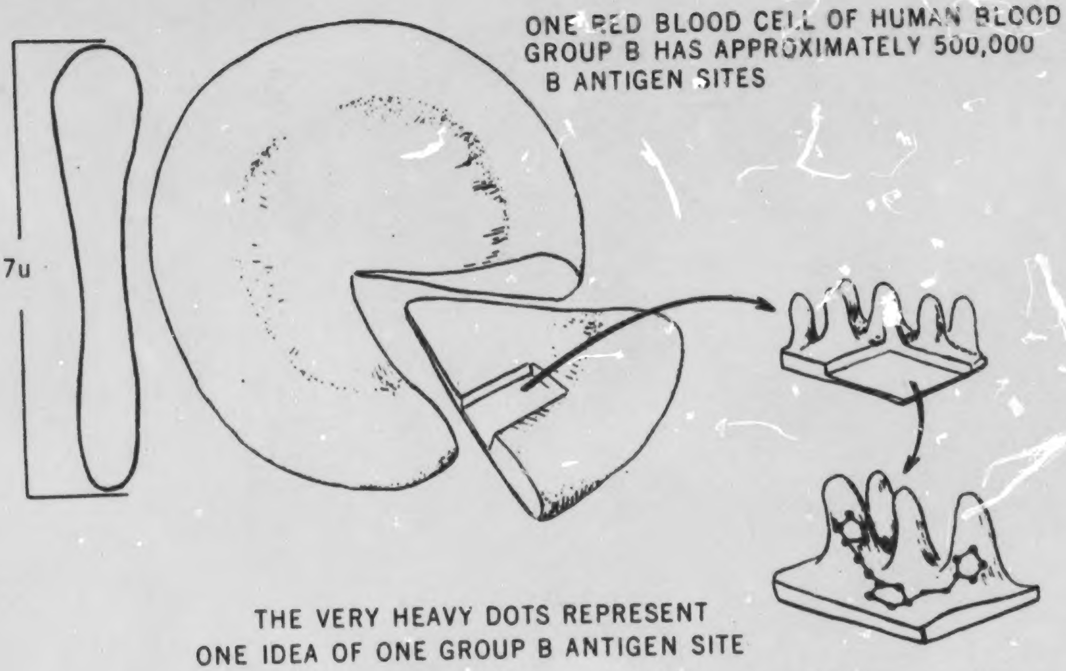

ONE RED BLOOD CELL OF HUMAN BLOOD GROUP B HAS APPROXIMATELY 500,000 B ANTIGEN SITES
7u
THE VERY HEAVY DOTS REPRESENT ONE IDEA OF ONE GROUP B ANTIGEN SITE

Figure 2. Antigenic determinants on a red blood cell.

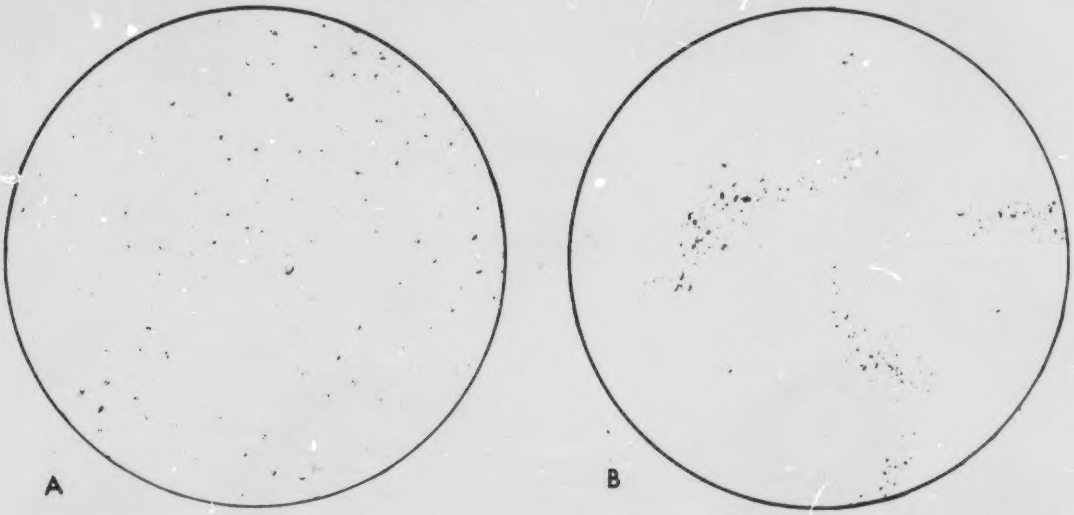
group AB father and a group A mother could have a group O child? Of course, they could not. On the other hand, it is never possible to prove that someone *is* the father of a particular child based on genetic studies. Other uses of genotyping in legal matters include the identification of blood samples associated with crime.

1-11. Determining genotype is sometimes essential in the location of blood for sensitized patients. For example, a female patient who has been sensitized against c (possesses antibodies against c antigen) should receive blood which is negative for the c antigen. In this case we phenotype using anti-c serum, and from this we derive the possible genotype. Further, one does not restrict the search for type-specific blood to patients who have been sensitized. Patients should never receive blood cell antigens that could sensitize (immunize) them. Keep in mind that c is usually most antigenic, next to D and D^u in the Rh series. Also keep in mind that *it is never wise to transfuse a female with blood from her husband.* She may be sensitized to antigens that are also possessed by the fetus if she should become pregnant.

1-12. **Immunology.** In the preceding course (CDC 90412) you were introduced to the terms "antigen" and "antibody." You learned that one term is usually defined with respect to the other. (Antigens are usually high molecular weight proteins, which elicit the response of antibodies.) For the purposes of blood banking, antigens and antibodies are best described at the molecular level. Antibodies are proteins that possess small molecular configurations on their surface, which are complementary to certain configurations on other proteins (antigens) whose presence caused the antibodies to be formed. You will learn later that substances other than proteins can also be antigenic. Most, if not all, anitbodies are gamma globulins. The complementary regions on the antibody are the antibody combining sites. There are at least two, and often many more, of these combining sites per antibody molecule. Sites on the antigen comprise a region called the antigenic determinant. Several molecules will link together at these complementary sites in a lattice arrangement during antigen-antibody reactions. Antigenic determinants on a red blood cell are hypothetically illustrated in figure 2.

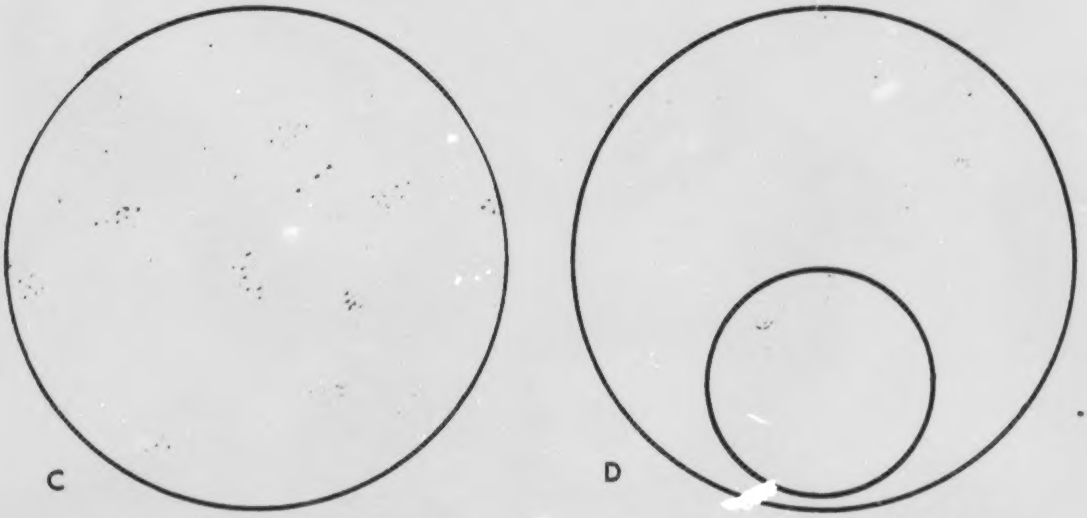
1-13. Antigen-antibody systems are extremely complex. Antibody molecules are not uniform with respect to combining sites. Some antibody combining sites are complementary to only a portion of the antigen determinant, while others may be complementary to the whole determinant.[2] In situations of this type, reactions encountered in blood banking may be weak and easily missed. Further, nonspecific agglutination may occur. Certain antibodies belonging to different systems may react at the same combining site. The traditional ideas that antigens must be "foreign" to the body is not strictly valid. In certain known diseases, autoantibodies are formed. For example, hemolytic anemia can result from autoantibodies to a person's own red blood cells.[1]

1-14. Antigens that are found in some members of a species but not in other members are called isoantigens. Likewise, immunization that occurs in response to an isoantigen is referred to as isoimmunization. Immunization due to blood transfusion is an example of isoimmunization. If an antigen is introduced into the body and the person does not possess the corresponding antibodies, there is usually no immediate reaction. About 2 weeks later, however, antibodies can be detected in the circulation if antigenic stimulation has taken place. (Some antigens apparently do not cause a measurable response.) If the person subsequently receives another dose of the same antigen, the immune response is more rapid, and the antibody titer will rise higher and remain at that level for a longer period. Antibodies due to a secondary response may also have greater combining properties with their specific antigens. It is interesting to note that the secondary response can be caused by antigens that are related but not necessarily identical to those that evoked the primary response. Isoimmunization is the reason it is often difficult to find compatible blood for a person who has received multiple transfusions over a period of time. Other causes of sensitization include complications of pregnancy (i.e., transfer of antigens from fetal to maternal circulation) and other



FREE UNAGGLUTINATED BLOOD CELLS

GROSS AGGLUTINATION OF BLOOD CELLS



WEAK AGGLUTINATION OF BLOOD CELLS

ROULEAUX FORMATION

Figure 3. True and false agglutination.

antigenic stimuli, including the injection of horse serum and other animal protein preparations. As far as is known, Rh antigens, specifically, may be introduced into a person only through transfusion or pregnancy.

1-15. *Antibody nomenclature.* Antibodies are classified in various ways. We can classify them as (a) immune and (b) natural, or naturally occurring. From what was said in the last paragraph about Rh antibodies, it would follow that they are immune antibodies. Anti-A and anti-B antibodies can be immune as well as natural. By "natural" we mean that antibodies so described are normally found in a person shortly after birth and throughout adult life and are not introduced by injections or pregnancy. It is theorized that ABO antigens are introduced into the body in food and in the air we breathe. Hence exposure to ABO-type antigens occurs continuously from birth; but it is not until several weeks after birth that anti-A and anti-B titers in the infant are high enough to be detected by the usual technique of proof grouping. This explains why a newborn infant may proof-group (back type) as group AB.

1-16. With respect to their effects on antigen-antibody reactions, antibodies have been categorized as complete and incomplete; univalent and bivalent; blocking antibodies; and first, second, or third "order" antibodies. Most such distinctions have fallen into disuse. The meaning of certain of these will be explained in terms of more modern concepts as we discuss specific forms of the antigen-antibody reactions in later chapters.

1-17. Today the nomenclature of antibodies is based more on chemical and physical properties defined by ultracentrifugation or electrophoresis techniques. In terms of their sedimentation rate during centrifugation at high speed, antibodies are often labeled in Svedberg (S) units. There is, of course, a relationship between molecular weight and the sedimentation constant. Most gamma globulins have a molecular weight of about 160,000, and they are found in the 7-S fraction. Blood group antibodies of the Rh, K, and Jk systems are usually 7-S globulins. A newer term for 7-Sγ globulins is γG or IgG. In general, "naturally occurring" antibodies of the Le, MNS, P, and certain other systems are macroglobulins of the 19-S variety. Synonyms for 19-S globulins are γM and IgM. These are the two principal groups of immunoglobulins, (IgG and IgM), of importance to the immunohemotologist. A third group, γA or IgA, are also 7-S immunoglobulins. These are implicated in the Rh system.

1-18. Variants of antigens are also commonly known. For example, there is more than one type of A antigen. The different variants of the A antigen are designated A_1, A_2, A_3, A_m, and A_x. Variants A_3, A_m, and A_x re relatively uncommon. Studies of group B and group O persons show that two distinct antibodies are common, namely anti-A_1 and anti-A_2 • A person of group A_2 may have anti-A_1. If a patient with anti-A_1 in his plasma is given group A_1 blood, there may be a transfusion reaction. Absorbed anti-A (anti-A_1) serum is available for identifying this subgroup. Another well known antigen is D^u , a variant of D antigen in the Rh system. We will discuss the variants somewhat further in Chapter 2.

1-19. *In vitro reactions.* The antigen-antibody reactions that a blood bank technician observes in the laboratory are of two general types, agglutination and hemolysis. Agglutination, or the clumping together of cells, is illustrated in figure 3, B and C. In reading agglutination it is necessary to rule out rouleaux. As you already know, rouleaux formation is a type of false agglutination in which the red cells stack together, as shown in figure 3,D. When rouleaux is observed macroscopically, the cells tend to tumble and slide when the glass slide or tube is tilted. True agglutinations are more granular and less regular then rouleaux. To eliminate rouleaux, add saline to the reaction mixture, one drop at a time, until the adhering cells disperse. False agglutination results from abnormal proteins or factors resulting from medication. We will mention the phenomenon of rouleaux again in treating sources of error associated with compatibility testing.

1-20. Hemolysis is often overlooked as evidence of antigen-antibody interaction. A poorly trained technician may have no reservations about using serum that shows evidence of hemolysis. Yet, if further hemolysis occurs because of antigen-antibody activity, the effect is masked and can be overlooked. While many other factors play a part, you should remember that hemolysis can result from antigen-antibody combination. In fact, some antibodies (e.g., anti-Lewis) tend to show hemolysis rather than agglutination.

1-21. Autoagglutination is the nonspecific clumping of an individual's cells by factors in his own plasma or serum. Autoagglutination is most common at low temperatures (e.g., 5°C), and therefore such antibodies are called cold agglutinins. You will see this pheomenon in primary atypical pneumonia and acquired hemolytic anemia, among other conditions. As a rule, cold agglutination is a reversible reaction. That is, when the test materials are warmed, the agglutination rapidly disperses. However, agglutination due to specific hemagglutinins does not disappear after warming.

1-22. Red cells agglutinate as the result of a two-phase process. First, antibodies combine with antigen molecules at specific sites on the cell surface. Second, the antibody-coated cells clump as they come into contact with each other. Agglutination proceeds faster if the contact of the cells is increased through centrifugation, stirring, or agitation. Thus, in laboratory test procedures, we centrifuge at specific times to force the cells together. False agglutination is a danger if the centrifugal force packs the cells too tightly, causing the suspension to give the appearance of clumping without an antigen-antibody reaction.

1-23. If fibrin or other enmeshing substances are present in a mixture of cells and serum, the red cells can become trapped and the particles have the appearance of agglutination. It is therefore important to carefully control the time and speed of centrifugation. The speed and time should be such that normal cells disperse readily; yet, centrifugation should be adequate to allow complete clumping when erythrocytes are mixed with very small amounts of specific antibody. Unusual or pronounced vibration of the centrifuge must be eliminated to avoid false readings.

1-24. In every antigen-antibody reaction there is an optimum proportion of antigen to antibody. When this optimum condition exists, complete and clearly visible reactions result. Weak or negative reactions occur whenever there is a significant excess of either antigen or antibody. In undiluted serum, the antibody content may be so great with respect to antigen that no clear-cut reaction can be seen. Occasionally, such a reaction is erroneously called negative. The true nature of the false-negative reaction is revealed when the serum is diluted. When a visible reaction fails to appear because of antibody excess, we refer to this as the "prozone" phenomenon. When the quantity of antigen greatly exceeds the quantity of antibody, we also see a false-negative or weak reaction. We call this a "postzone" phenomenon. You can correct the quantitative relationship between antigen and antibody by serially diluting the antigen or antibody mixture used in the test.

1-25. *Complement.* A thermolabile substance (complement) found in normal serum is both necessary and responsible for the hemolysis of red cells in antigen-antibody reactions. Complement is believed not to increase during immunization. Various antibodies fix complement but some do not. Antibodies that fix complement include anti-A, anti-B, anti-Le^a, anti-Le^b, anti-Jk^b, anti-K, anti-Fy^a, and some others. However, antibodies of the Rh system do not usually fix complement. At temperatures lower than 37° C., complement-fixation occurs with cold "incomplete" anti-H. This can result in hemolysis of cells exposed to temperatures below 37° C. Complement is also necessary in this case for the antibody to combine with the antigen. The term "incomplete antibody" denotes an antibody that adheres to the surface of red cells suspended in saline but fails to agglutinate them. This term was more common in early blood banking literature. *In vivo* antibody coating and/or hemolysis may result from complement-fixation. The use of an enzyme in treating cell preparations may alter certain antigens, e.g., K and Fy^a, and thus alter complement-fixation properites so that test results are unpredictable.

1-26. We know that some antigen-antibody reactions are complement-dependent. This can be demonstrated by studies of complement inactivation. It can also be easily shown in the laboratory that most human blood group antibodies do not hemolyze untreated red cells *in vitro*. When the hemolysis of untreated cells does occur, complement-fixation is a possibility. Actually, the importance of complement in blood-banking is best illustrated *in vivo* because survival times of erythrocytes correlate closely with the presence or absence of complement-binding antibodies. Cells coated with complement-fixing antibodies are removed from the circulation much faster than cells sensitized with noncomplement binding antibodies.

2. Rh Isoimmunization

2-1. Rh isoimmunization is of special concern to the clinical laboratory because it is the most common cause of hemolytic disease of the newborn (HDN, referred to in earlier literature as eryththroblastosis fetalis). This fact has always involved the laboratory of hospitals with an obstetric service.

2-2. Rh typing and antibody studies are a necessary part of the workup in known and suspected cases of HDN, both during pregnancy and after birth. The direct Coombs procedure and its relevance to this problem are well known to any laboratory technician who has performed the Coombs test on cord blood at all hours of the day and night. The blood bank technician who secures and prepares blood for exchange transfusions is also keenly aware of the impact the so-called Rh problem has on the technician.

2-3. Since 1965, the whole clinical subject of diagnosing and preventing HDN has taken on new aspects. It was discovered that injecting a concentrated solution of antibodies into the mother can destroy Rh positive cells, which enter the mother's circulation from the fetus, before her antibody-producing cells respond to the presence of the infant's antigens. This was a tremendous advance in the area of Rh disease. We will discuss the use of the concentrated antibody solution, anti-Rh$_0$ (D) immune globulin (RhoGAM®),* later in this section. First let us briefly review the immunology of Rh isoimmunization.

2-4. **Immunology of HDN.** Hemolytic disease of the newborn is characterized by the destruction of an infant's red blood cells by specific antibodies, which were transferred across the placenta from the mother. The disease actually begins *in-utero* and may result in the death of the fetus. Often it is not a serious threat to the well-being of the infant until shortly after birth. As red cells are destroyed, the infant becomes jaundiced, and the oxygen-carrying capacity of the erythrocytes that remain functional may become inadequate. In this case the infant suffers brain damage and possible death.

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*RhoGAM®—registered trademark of Ortho Diagnostics, Raritan, New Jersey

2-5. The production of antibodies by the mother may be in response to almost any antigen. However, the Rh antigens most often responsible for isoimmunization in pregnancy are D and c. ABO incompatibility can also present a problem. The immunoglobulin, identified as I$_g$G or (γG 7-S), is transferred to the fetus via the placenta. This means that antigens which are not I$_g$G (e.g., anti-M, -N, -P$_1$, Le^a, and Le^b) rarely, if ever, cause hemolytic disease of the newborn.[4] Previous pregnancies and blood transfusions possessing the same "foreign" antigen vastly increase the chances for a significant antibody titer in later pregnancies.

2-6. You can readily see from your knowledge of genetic inheritance how a D negative mother and a D positive father can produce D positive offspring. It should also be apparent to you at this point why table 1, discussed earlier, is of importance to the practical as well as to the theoretical blood banker.

2-7. If hemolytic disease of the newborn develops, the only effective treatment includes an exchange transfusion. The purposes of an exchange transfusion are to:

a. Replace destroyed erythrocytes.

b. Remove antibodies to the infant's red blood cells.

c. Remove some small amount of bilirubin, but mainly to prevent a further rise in bilirubinemia. (In severely affected infants, the large amounts of fixed bilirubin present in tissues, aside from circulating nonfixed bilirubin, cannot be markedly influenced by exchange transfusion.) It is interesting to note that serum albumin has an affinity for binding bilirubin and is, therefore, of therapeutic value. Though still at the research level, only the so-called nonfixed bilirubin is believed to have a toxic action on brain cells. If this is the case, it has been suggested that albumin-binding bilirubin rather than direct and indirect serum bilirubin be used as an index of the requirement for transfusion.[5]

2-8. Physicians perform exchange transfusions by alternately withdrawing blood from the infant and replacing it with an equal amount of fresh donor blood. This is done in 10- to 20-cc. increments until 300 to 600 cc. of blood is replaced, depending on the size of the infant. The exchange is usually carried out through the umbilical vein, though other vessels may be used. In selecting the type of blood for exchange transfusion, the most important consideration is that the fetus produces little or no natural antibodies; hence, most of the circulating antibodies present at birth are from the mother. Accordingly, any blood administered must be compatible with the mother's serum. Recently published articles emphasize the fact that although exchange transfusion deaths occur more frequently in cases of severe hemolytic disease, the mortality rate in full-term infants is negligible.[6] The overall risk is 1 to 2 percent.

2-9. The cardinal rules governing the choice of blood for exchange transfusion are:

- Avoid the antigen responsible for the anitbodies present in the mother's serum.
- Administer blood with phenotype specific for the mother as to Rh, Kell, etc.
- Use group specific cells compatible with the mother and child or group O with low-titer serum.
- Use fresh blood, preferably under 24 hours old, and never more than 72 hours old unless its use is absolutely necessary to avoid delaying an exchange transfusion.

2-10. When the mother and infant are of compatible blood groups, e.g., mother and infant are both group A, then group A negative blood is preferred by most clinicians. In other cases, e.g., the mother is group A and infant is group B, it is desirable to use low-titer group O negative blood. By "low titer," we usually mean a titer of anti-A and anti-B less than 1:50. Remember that blood selected for exchange transfusion *must be compatible with that of the mother.*

2-11. **Rh$_o$(D) Human Immune Globulin (RhoGAM®).** RhoGAM® is a relatively new biological, Rh$_o$ (D) (human) immune globulin, which is approved by the Food and Drug Administration and is currently available. This product represents a significant breakthrough in the prevention of maternal isoimmunization. It promises to eliminate Rh hemolytic disease of the newborn. Use of this substance is expected to save approximately 10,000 infants annually in the United States.

2-12. Rh$_o$ (D) (human) immune globulin is a concentrated solution of anti-Rh$_o$ (D) in high titer (1:250). A minor crossmatch is required because of the need to confirm compatibility with the maternal cells. Routinely detectable antibodies other than anti-D are in relatively low titer to meet the NIH licensing specifications. Since the solution used for the crossmatch is already dilute (1:1000), thus simulating the *in vivo* dilution of the therapeutic dose, these other antibodies should not ordinarily constitute a problem. Nevertheless, prudence, as well as established procedure, dictates that careful blood banking techniques be used. If one vial is incompatible, another lot may be acceptable.

2-13. Rh$_o$ (D) (human) immune globulin is useful only in women not previously isoimmunized. It must be administered within 72 hours after delivery to be optimally effective. Women selected to receive Rh$_o$ (D) (human) immune globulin must meet the following criteria:

a. The mother must be Rh$_o$ (D) negative and D^u negative.

b. The mother must not have circulating anti-Rh$_o$ antibodies.

c. The infant must be Rh_o positive or D^u positive.

d. The direct Coombs test performed on the infant's cells should be negative, but in many cases of ABO incompatibility and other situations the Coombs test may be positive. It must be definitely known that the positive Coombs test is *not* due to Rh isoimmunization.

2-14. ABO differences between the mother and infant can cause a positive Coombs—this should not be considered an absolute contraindication to the use of RhoGAM®. Other causes of positive Coombs [illegible] common, and, unless due to the presence of anti-Rh_o, are not pertinent. The mother's serum may give positive Coombs reactions from causes other than the presence of circulating anti-Rh_o. When a question exists regarding the cause of a positive antibody screening test, the presence or absence of anti-Rh_o should be confirmed by tests with a panel of known cell types.

2-15. Legal liability is possible if isoimmunization develops in the mother as a result of failure to administer RhoGAM® in the absence of contraindications that may arise from patient or laboratory considerations. This product is not to be administered to the infant, since its purpose is to destroy Rh positive cells in the maternal circulation. If administered to the infant, a hemolytic reaction is to be anticipated.

2-16. Evidence exists that a small percentage of women become isoimmunized at the time of spontaneous abortion. It is also possible for isoimmunization to occur during an ectopic pregnancy. RhoGAM® is often administered to patients with abortion or ectopic pregnancy if they otherwise satisfy the criteria listed previously, except that blood studies cannot be performed on the fetus. In these cases, it must be assumed that the fetus is Rh_o positive or D^u positive.

2-17. Mothers giving Rh negative reactions should be tested with a second manufacturer's anti-D reagent antiserum and Coombs reagent (anti-human globulin) in order to exclude false-negative reactions. This testing should be performed as early as possible in the pregnancy and repeated at delivery before the Rh_o (D) (human) immune globulin crossmatch, as a patient identification check.

2-18. Some physicians suggest that positive controls could be incorporated by (a) testing a small amount of the prediluted RhoGAM® against known D positive cells and (b), after administration of the therapeutic vial of RhoGAM®, testing "the last drop" in the vial against known D positive cells. If the antibody solution agglutinates the known D cells, the product can be considered satisfactory.

2-19. RhoGAM® should be stored under the same conditions used for whole blood; that is, 4° to 6° C. under controlled conditions, with maintenance of a continuously recording thermometer. A proper blood bank refrigerator should be available for any facility performing crossmatching. The storage period depends on this control. Freezing is prohibited. Detailed information on instructions for use, control, and procurement, as well as laboratory guidance regarding this pharmaceutical, is provided by the manufacturer.

Footnotes

1. P. L. Mollison, *Blood Transfusion in Clinical Medicine* (4th ed., Philadelphia: F. A. Davis Co.), p. 184.
2. Elvin A. Kabat, *Structural Concepts in Immunology and Immunochemistry* (New York: Holt, Rinehart, and Winston, Inc., 1968), p. 7.
3. *Ibid.*, p. 10.
4. Mollison, *op. cit.*, p. 644.
5. "A Criterion for Exchange Transfusion. The Binding Capacity of Serum Albumin. *(Un critere pour les exsanguino-transfusions. La capacite de fixation de l'albumine du serum),*" *Concours Med.* (1968), 90, 16 (3393-3397).
6. D. J. Frank and R. Miraflor, "Exchange Transfusion. A Safe Procedure for Most Newborns." *Ohio St. Med. J.* (1968), 64, 6 (714-16).

CHAPTER 2

Blood Group Systems

IT WAS THE YEAR 1900, and the gas lights burned late into the night in the modest laboratory of a young American pathologist, Dr. Karl Landsteiner. The experiments Dr. Landsteiner performed were to solve many of the mysteries of blood that had clouded men's minds for centuries.

2. This scientist mixed the red blood cells of a sick individual with the serum of another sick individual. To his amazement he noticed that the red blood cells of the first patient were forming into clumps. He repeated his mixing of serum and erythrocytes with other patients. It soon became apparent that a reaction was taking place in a rather observable manner and following some sort of a pattern.

3. Landsteiner wondered if his observations were related to a disease process. To test his results, he drew blood specimens from himself and his fellow workers. Once again he mixed erythrocytes from one person with the serum of another person and observed the same clumping reaction that he had seen in his previous experiment with sick patients. The phenomenon appeared to be a natural one; it was demonstrated in both normal and sick people. The experiment continued with some refinements. He began to mix one person's erythrocytes with several different human sera in an individual series of steps. After a considerable amount of research, Landsteiner and his assistants were able to categorize blood into three groups (A, B, and O) according to its agglutination properties. A year later, von Decastello and Sturli found the fourth group which they called AB.

4. In 1940, Landsteiner and another American, Alexander S. Wiener, discovered the RH factor through experiments with rabbits and the Rhesus monkey. This led to further work in the development of the RH system, which was found to be independent of the ABO system of classification. A year after Landsteiner's discovery, Philip Levine, together with Burnham, Katzin, and Vogel, identified the Rh factor clinically in a case of hemolytic disease of the newborn. At the same time, the Rh factor was discovered independently by Paul Moreau, who worked in Occupied Belgium.

5. In this chapter we will explore some of the modern concepts associated with blood groups as they are currently identified.

TABLE 2
"NATURALLY OCCURRING" ANTIGENS AND ANTIBODIES OF THE ABO BLOOD GROUPS

Blood Groups	Antigen on the Erythrocyte	Antibodies in the Serum
A	A	Anti - B
B	B	Anti - A
AB	Both A and B	Neither Anti-A nor Anti - B
O	Neither A nor B	Both A and B

TABLE 3
APPROXIMATE INCIDENCE OF BLOOD GROUPS IN THE UNITED STATES

Population	Group A	Group B	Group AB	Group O
Caucasians	44%	9%	4%	43%
Negroes	27%	17%	4%	52%

3. The ABO and Lewis Groups

3-1. At this point in your career, you are quite familiar with the ABO blood group system, which Landsteiner discovered. Perhaps you are less familiar with the Lewis system discovered in 1946 by Mourant. We will consider both of these systems in this section because the chemistry of Lewis and of ABO blood group substances is similar.

3-2. **ABO System.** From our discussion in Chapter 1, it is quite evident that there are antigens labeled "A" and "B" located on the red blood cells. If both antigens are present, the person is in blood group AB; and if neither antigen is present, he is in group O. It is also elementary that people possess antibodies in their serum for the antigen that is lacking on their red blood cells, as enumerated in table 2.

3-3. *General.* Antibodies are the result of antigenic stimulation, and it is the antigens, not the antibodies, that are under direct genetic control. "Naturally occurring," as defined in Chapter 1, means that such antibodies are produced naturally, without injection or pregnancy, by natural exposure of the body to substances with antigenic properties identical, for all practical purposes, to those of the A and B antigens.

3-4. The approximate incidence of the various blood groups in the United States is given in table 3. Group O is certainly the most common, and blood of this group is sometimes called the universal donor blood because of the absence of A and B antigens on the red cells.

3-5. Referring to figure 4, you will note that A and B antigens develop from an H substance. This

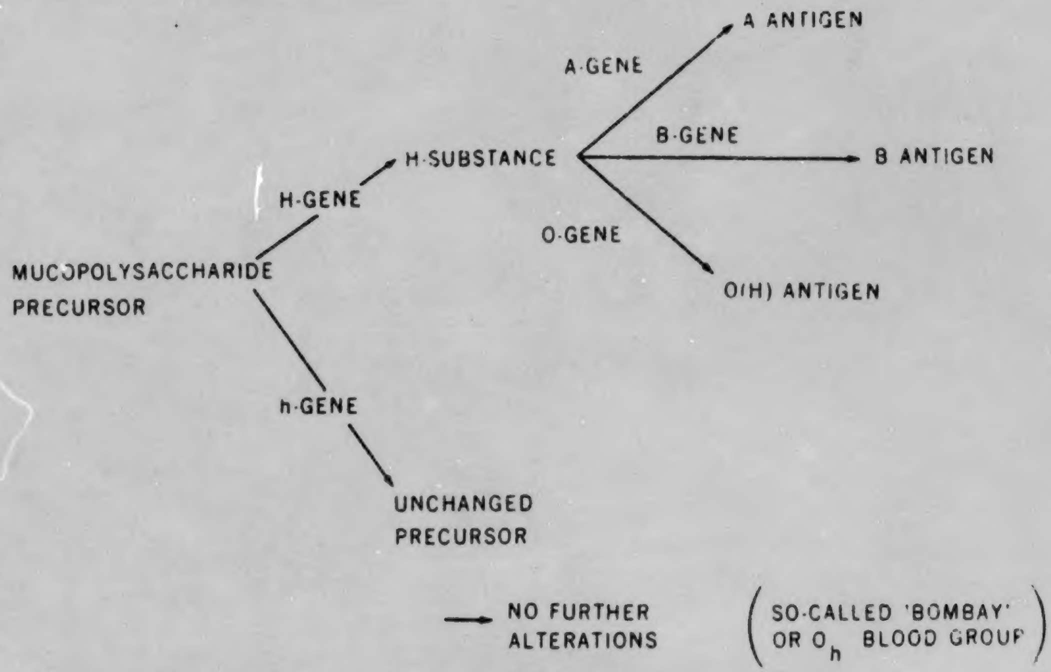


Figure 4. ABO antigen development.

precursor is transformed into specific A or B substances under genetic influence. The O gene does not change the H substance; therefore, group O cells contain more H substance than cells of other groups. If the H substance is not transformed into either A or B, the red cell contains the H substance rather than the specific A or B antigen (or both). The H substance is not a product of the O gene, but a precursor of A and B antigens developed from a mucopolysaccharide. The O gene does not produce a recognizable effect and because of this is sometimes referred to as an amorph. Blood groups other than O have lesser amounts of H substance and can have anti-H antibody. There is a rare kind of blood group O called O_h or Bombay blood group. (The term "Bombay" is now becoming obsolete.) The blood of individuals in group O_h contains no H, A, or B substance. They have anti-H as well as anti-A and anti-B in their serum.

3-6. Antigen variants of group A were mentioned in Chapter 1. Subgroups of B are not known in the same sense as the subsgroups of A. However, weak B antigens are sometimes found; or, what is just as important, they are sometimes overlooked. Weak group B antigens can be detected by elution methods or the use of plant agglutinins called lectins. These agglutinins are globulins with a structure simpler than plant antibodies. Lectins have a great affinity for specific red cell antigens and can thus be used as a sensitive means for their detection.

3-7. Several cases are reported in the literature of so-called acquired B-like antigens. These are cases in which the red cells react with anti-B grouping sera, but in which the serum also contains anti-B antibodies. Situations of this type are quite uncommon, but nonetheless they have been found. Evidence suggests that the B-like antigen is the transformation product of a red cell antigen caused by the action of bacterial enzymes.

3-8. It may surprise you to learn that A and B antigens have been demonstrated on leukocytes, platelets, and other cells besides erythrocytes. This fact has limited practical significance at the present time, but may become more important as blood banking techniques become more refined. A practical application of this phenomenon is in rape cases where seminal fluid is compared with semen from the suspect. The rationale of testing body fluids other than blood is based on the fact that A and B substances are secreted in the body fluids of most individuals. The ability to secrete is controlled by the Se gene.

3-9. *ABO grouping*. Because of the permanent, reliable characteristics of blood group antigens and the simplicity of technique, blood groups are routinely identified by direct cell grouping. This can be done on a microscope slide or in a tube. Generally speaking, tube methods are more reliable. You must be careful to follow the instructions furnished with the grouping or typing antiserum you are using. Correctly used, the term "typing" is limited to identification of Rh antigens, whereas "grouping" applies to other antigen systems. Some antisera are used with saline-suspended cells, while other antisera must be used with a serum or protein suspension. Reasons for this will be clearer as you study later in this volume the necessity of a high protein medium. Slide methods are recommended only for screening procedures or identification for administrative purposes, e.g., to be used on ID cards. Blood for transfusion should always be tested by the tube method. There are many possible errors in forward (direct) grouping. Antisera are sensitive biologicals that can become contaminated, impotent, or otherwise unsuitable. The technician may be confused by misleading reactions due to cold autoagglutinins, saline reactive agglutinins other than A and B, and polyagglutinable erythrocytes. Reactions caused by subgroups, clerical mistakes, haste in reading, and reading over a warmed surface, are also possible causes of error in direct cell grouping.

3-10. To supplement forward grouping, serum grouping is an essential quality control procedure. It

TABLE 4

INTERPRETATION OF SERUM GROUPING REACTIONS (GROUP O CELLS SHOULD BE USED AS A CONTROL)

Blood Group	A Cells	B Cells
A	No agglutination	Agglutination
B	Agglutination	No agglutination
AB	No agglutination	No agglutination
O	Agglutination	Agglutination

is also called proof grouping, reverse grouping, or backgrouping, and the procedure for serum grouping is found in AFM 160-50. The American Association of Blood Banks stresses that serum grouping should be performed on all recipients and donors. Infants less than a few months of age may not have developed sufficient anti-A and anti-B to proof-group correctly.

3-11. By the use of known A and B cells, serum grouping reactions can be interpreted as outlined in table 4. There are good reasons for using A_2 and O cells as well as the A_1 cells recommended in AFM 160-50. The reason for using A_2 cells is to prevent misidentification of A_2 as group O. (A_2 and A_2B persons may have anti-A_1 in their sera.) Ordinarily, this possible discrepancy would be brought out in front grouping because most anti-A grouping serum detects A_2 as well as A_1. We are suggesting, however, that in some cases the use of A_1 cells by themselves could "confirm" a subgroup of A erroneously forward-grouped as O. This would be a double error.

3-12. The use of group O cells in serum grouping provides a control measure. If agglutination is due to a cold agglutinin, the group O cells would also agglutinate. Prepared suspensions of cells for serum grouping are available commercially. If you prepare your own cell suspensions, you should take extra care in proper group identification. Red blood cells suspended in saline may undergo rapid deterioration. It is essential, then, that you use the cell suspensions as soon as possible after preparation and certainly no later than the day of preparation.

3-13. **Lewis Goups.** The Lewis blood system has been of particular interest to immunologists because of its relationship to the salivary secretion of blood group specific substances. The anti-Lewis agglutinins designated anti-Le^a and anti-Le^b occur naturally but quite infrequently in human serum. These antibodies, when present, are usually nonreactive at body temperature and exist in low titer. Transfusion reactions involving Le^a incompatibility have been reported.

3-14. Lewis antigens are thought to occur in saliva and in the serum, from which they are absorbed by the red blood cell. Nonsecretors have the phenotype Le (a+b−). Persons who inherit the Lewis gene but are secretors have the phenotype Le(a−b+). These phenotypes are referred to as Le^a and Le^b, respectively. The presence of A_1 may interfere with the expression of Le^b. Various involved relationships between the ABO system and the Lewis system have been postulated. The erythrocytes of Le^b positive individuals are agglutinated by anti-Le^b serum. Cells of the A_1 subgroup, group B, or A_1 + B, agglutinate only with certain Le^b antisera. This leads to the conclusion that only group O blood has a significantly detectable amount of the Le^b antigen.

3-15. Cells of the Le^a group are presumable all agglutinated by Le^a antiserum. Further, Le^a positive red cells have no Le^b antigen. Reactions to Lewis antigens during the first 15 months of life change. Some people are negative for Le^a and for Le^b. About 70 percent of the population is Le^b positive, and about 25 percent are Le^a positive. The rest are negative for both Le^a and Le^b.

3-16. The anti-Le^a antibody is found occasionally in genetically Lewis-negative individuals. The presence of anti-Le^a can be demonstrated with commercially available Le^a positive cells. Anti-Le^b is found even less often than anti-Le^a. Lewis antibodies are "naturally occurring," because they are found in individuals who have received no known antigenic stimulation except, of course, through their natural environment. Clinically, Lewis antibodies can cause hemolytic transfusion reactions, though this is rare. The Lewis group is rarely associated with hemolytic disease of the newborn. A few such cases have been reported, but the problem is by no means common. Le^a antibodies are small I_gG immunoglobulins which can enter fetal circulation, whereas Le^b antibodies are of the large I_gM type.

4. Rh and Other Systems

4-1. The subject of Rh blood groups has been one of great interest since Levine and Stetson described their now famous clinical case of HDN in 1941. We have already discussed Rh isoimmunization in Chapter 1. Let us now study the Rh system as a taxonomic grouping. In concluding this section we will also identify some of the other blood group systems, including the Kell, Duffy, Kidd, MNS, and a few miscellaneous classifications.

4-2. **The Rh System.** A basic group of six closely related antigens makes up the Rh system. Fisher and Race have designated these as D and d, C and c, and E and e. The corresponding antibodies were designated by Wiener as anti-Rh_0, and anti-Hr_0, anti-rh′ and anti-hr′, and anti-rh″ and anti-hr″, to designate allelic genes in a way that is not completely comparable to the Fisher-Race system. Each factor, except d (Hr_0), has been identified by a reaction with its specific antibody, produced in individuals who have been immunized. The antibody whose formation is stimulated most frequently is anti-D (anti-Rh_0), which reacts with the erythrocytes from 85 percent of the white U.S. population and, in so doing, reveals D (Rh_0), the originally described Rh antigen.

4-3. Contrary to earlier information, anti-d typing serum is not available, commercially or otherwise. The term "Rh positive" commonly refers to D (Rh_0) positive blood. This is a somewhat dangerous simplification of terminology. A blood donor, for example, is no longer considered Rh negative by most authorities unless he is also negative for C, D, E, and D^u. Red cells that contain the D^u factor are not usually agglutinated by anti-Rh$_o$ (D) serum. D^u bloods give positive reactions only after being exposed to anti-Rh$_o$ (D) antibodies and then tested with antihuman serum by the indirect Coombs technique. Some anti-Rh$_o$ (D), CD, or CDE antisera detect this antigen but many otherwise potent antisera do not. For this reason, all D negative blood donors must be further tested by the indirect Coombs technique.

4-4. Test cells are incubated with anti-D at 37° C. for 1 hour and washed three times with saline. Run Coombs positive and negative control cells to check the effectiveness of your Coombs serum. Keep in mind that Coombs positive blood will type positive for D^u and, therefore, this is not a valid test with blood that is Coombs positive for a reason other than presence of D^u. Coombs serum is added, and the cells are centrifuged and examined for agglutination or hemolysis. The American Association of Blood Banks (AABB) requires that all blood banks seeking its accreditation perform the incirect Coombs test for D_u identification. The fourth edition of *Technical Methods and Procedures of the AABB* states that a person who is D^u positive is Rh positive, whether a patient or donor.[1] This position is not fully accepted by many workers in the field. According to Mollison, D_u positive people do sometimes form anti-D.[2] It would, therefore, seem unwise to give D positive blood to a D^u positive, D negative recipient. The D^u variant occurs more often in Negroes than in Caucasians.

4-5. Other Rh antigens besides those already mentioned are known. Included are the V antigen discovered in 1955, the G antigen described in 1958, and the LW (Landsteiner-Wiener) antigen shown in 1964 to be genetically independent of other Rh genes. It is now thought that LW is the precursor substance for Rh groups and is analogous to H substance in the ABO system. Finally, it is worth pointing out that rare cases have been described in which the red cells were lacking in some or all of the RH antigens. In the latter instance, action of a suppressor gene is suspected in the case of a parental genotype "normal" with respect to the Rh system.

4-6. **Other Blood Systems.** Human erythrocytes possess many known antigens. Since antigens are molecular complexes of relatively small size, thousands of them may be present on the surface of a red cell. In addition to ABO and Rh, some of the other clinically important antigens include Kell, Duffy, and Kidd. Blood antigens such as M, N, S, s, k, P, Lutheran, and Lewis are important in the exact identification of individual blood specimens. There is little doubt that these lists will expand as research in the field of immunohematology progresses. Blood bank technicians should be familiar with the most important of these blood antigens. A brief discussion of their occurrence, makeup, and detection is presented in the paragraphs that follow.

4-7. *Kell system.* The Kell blood system includes the antigens K and k. Approximately 90 percent of all Caucasians lack the K factor, and sensitization to this antigen has been an occasional cause of hemolytic transfusion reaction and hemolytic disease of the newborn. Inheritance of the Kell blood antigens is determined by two gene alleles, K and k. All individuals who are homozygous kk or who are heterozygous for K together constitute about 98.8 percent of the population. As a result of the rarity of homozygous KK individuals, sensitization to this factor is extremely uncommon. With the use of two sera, anti-K (Kell) and anti-k (cellano), it is feasible to determine an individual's specific Kell genotype. Anti-K shows a dose response that may be useful in the detection of heterozygous individuals. Anti-K is almost always of the incomplete variety of antibody and is usually most reactive in the indirect Coombs test. Examples of anti-Kell, which react exclusively with the enzyme method, have been reported. Other Kell variants have been described.

4-8. *Duffy system.* The two antigenic factors comprising this system are designated Fy^a and Fy^b. They are inherited through a pair of gene alleles also named Fy^a and Fy^b, respectively. Anti-Fy^b serum is quite rare and, as a result, the presence of Fy^a accounts for the two blood types ordinarily recognized in the Duffy system. The Duffy positive and negative types determined by the use of anti-Fy^a serum are referred to as Fy^a positive (a+) and Fy^a negative (a−). The Fy^a negative type occurs in about 35 percent of the Caucasian population; and, therefore, immunization against Fy^a is an occasional cause of hemolytic transfusion reaction. The anti-Fy^a antibody can sometimes be recognized only with the use of antihuman serum. The albumin or trypsin methods are, as a rule, quite inadequate.

4-9. *Kidd system.* The antigenic factors of the Kidd system are designated Jk^a and Jk^b. Kidd isoimmunization due to the Jk^b antigen is very infrequent, and most of the cases reported involve Jk^a. The blood bank technician is generally concerned with two blood types in the Kidd system. These are Jk^a positive and Jk^a negative, as determined by anti-Jk^a serum. Jk^a negative occurs in about 25 percent of the population, and sensitization to Jk^a positive cells occurs occasionally as a consequence of pregnancy.

4-10. *Lutheran system.* The antigens in the Lutheran blood system are designated Lu^a and Lu^b. These factors are not common in transfusion reactions. The incidence of the Lu^a type is about 8 percent. There is evidence that the Lutheran system may be linked with the previously mentioned Lewis system.

4-11. *P system.* The P system has been investigated and implicated in transfusion reactions. Recently discovered complexities in this system have prompted a change in its antigen nomenclature. The P positive cells of the old classification are now regarded as P_1 and the P negative as P_2. A third extremely rare gene, p, was formerly classified as Tj negative. These individuals have antibodies that react with both P_1 and P_2 cells. The P_1 antigen is present in 75 percent of Caucasians and 95 percent of Negroes. Anti-P_1 is present in two-thirds of the people with P_2 and is a naturally occurring antibody reacting at low temperatures.

4-12. **Rare Antigens.** There are certain blood antigens that have a very infrequent occurrence in random or unrelated blood specimens. These factors are, therefore, referred to as "private" or family antigens. These blood antigens include the following: Levay, Gr, Jobbins, Mi^a, Becker, Ven, Ca, Be^a, Wr^a, Di^a, By^a, Vw, and Chr^a.

4-13. In contrast to these private or family antigens, there are high incidence or "public" antigens. The latter were discovered through antibodies that react with the red cells of most of the population. Although these antibodies are rare, they can create great difficulties in crossmatching because of their capacity to react almost universally. Examples of public or high incidence antigens are: Vel (Ve^a), Cartwright (Yt^a), Gerbich (Ge), Auberger (Au^a), I-i, and Sm., Kp^b, and Js^b. In the bibliography of this volume, we have listed some of the references you will find valuable in seeking additional information on these and other antigens.

Footnotes

1. *Technical Methods and Procedures of the American Association of Blood Banks* (4th ed., AABB Chicago), p. 73.
2. P. L. Mollison, *Blood Transfusion in Clinical Medicine* (4th ed.; Philadelphia: F. A. Davis Co.), p. 291.

CHAPTER 3

Blood for Transfusion

THE TRANSFUSION of blood did not become an accepted and effective medical practice until about 40 years ago. Yet, throughout history attempts were made to transfuse blood. One of the best known attempts in earlier times took place in 1490. A physician tried to rejuvenate Pope Innocent VIII by getting him to drink the blood of three youths. According to historical accounts, the Pope died, the donors died, and the attending physician fled the country.

2. On 15 June 1667, Jean Baptiste Denys (1625-1704) transfused a boy with blood from the artery of a lamb. Later in 1667, Richard Lower (1631-1691) performed two direct transfusions from a sheep to a man. Then in 1668 one of Denys' patients who had been transfused died, and the resulting legal action discouraged further experimentation.

3. The first successful therapeutic transfusion is usually credited to a 19th century physician, James Blundell (1790-1877). After concluding that only human blood should be transfused to humans, and after treating nearly 20 "hopeless cases" unsuccessfully, Blundell transferred 8 ounces of blood from his assistant to a woman suffering postpartum hemorrhage.[1] It is doubtful how much value 8 ounces of blood could have been, but Blundell wrote in 1829, "Although the patient received only 8 ounces in 3 hours, she felt as if life were infused into her body."

4. Unlike James Aveling (1821-1892), the inventor of a portable transfusion outfit, the blood bank technician of today does not carry a transfusion kit around hoping for a suitable case. Rather, he is a sophisticated technician in a modern, well-equipped laboratory. Let's look into his laboratory now and view some "tricks of the trade" unknown to his distinguished and not-so-distinguished predecessors.

5. Specific Blood Banking Procedures

5-1. After what we have said in Chapters 1 and 2, it would be an understatement to say that blood is a complicated, immunologic substance. The practical blood banker makes use of the accumulated knowledge of antigens and antibodies, and both provides valuable diagnostic data to the physician and also makes blood a safe product for transfusion. In this section we will elaborate on particular laboratory tests closely associated with blood banking.

Coombs Test. An observable reaction cannot occur between an antigen and its corresponding antibody unless both are suspended in a suitable medium. Certain physical factors prevent the binding process. Theoretically, two of the most significant factors that keep antigens and antibodies apart are (1) repelling electrical forces among the colloidal particles and (2) the size of the molecules, or specifically the dimensions of the antibodies considered.

5-3. One of the media used to enhance and permit the interaction of certain antigens with their respective antibodies is anti-human γ-globulin (AHG). It was adopted for this purpose in 1945 by Coombs, Mourant, and Race. As you know, AHG is commonly called Coombs serum. The way in which AHG acts is illustrated in foldout 1 in the back of this volume, which describes the direct (detail A) and indirect (detail B) Coombs test. The only significant difference between the direct and indirect Coombs test is that, in the direct Coombs test, we are concerned with cells which have been coated with antibody *in vivo;** In the indirect Coombs test, we are testing serum for Coombs-reactive antibodies. In the past, the term "immune incomplete antibody" was often used in describing AHG-reactive antibodies. However, there are other antibodies that fail to react in either saline or anti-γ-globulin serum, and they are also called by the imprecise term "incomplete antibodies." There are also IgM antibodies (e.g., Lewis) that must be detected with anti-IgM globulin serum or by complement-binding techniques. Thus you can easily see that what is detected with Coombs serum depends upon the specificity of the Coombs serum you are using. Most available antihuman serum contains both anti-γ-globulin fractions and anti-non-γ-globulin fractions.

**In vivo* sensitization refers to sensitization which occurs in the blood stream. It may also occur *in vitro* as a result of intentionally mixing antisera and cells in the laboratory.

A broad spectrum Coombs serum should also contain anticomplement (C') complement-binding antibodies.

5-4. Coombs serum can be prepared in a number of different animals. The most popular are rabbits, goats, and sheep, selected on the basis of handling ease, good immunization response, and the lower costs involved with these domestic animals. The principles of AHG production are essentially those with which you are familiar. The antigen is human globulin, which is foreign to the animal. The antigen is fractionated from human plasma. The human gamma globulin stimulates the animal to produce an antibody, antihuman globulin. After the antibody has been produced in a rabbit, the collection of rabbit serum can be continued for years if periodic stimulating doses of antigen are given. Since each rabbit's response to the antigen is different, it is necessary to blend and standardize the resulting antihuman globulin reagent. To be of commercial worth, the antibody must be of high titer so that it can be diluted and still sold as an effective reagent.

5-5. When you perform a Coombs test, it is very important that you wash the test cells completely free of globulin. At least three washings are necessary to accomplish this. Further, you should *never* use your finger or your thumb to stopper the tube when you are mixing the cell suspension. By so doing you may introduce extraneous human globulin. This could result in a negative Coombs test because the globulins from body secretions neutralize the antihuman globulin of the Coombs serum. Use a rubber stopper or paraffin film when mixing cell suspensions. Commercially prepared control cells or saline-washed, anti-D coated, group O (Rh positive) cells should be used. Preservation is possible with modified Alsever's reagent. Commercial preparations are by far more reliable. Always run controls with every test. Do not overcentrifuge the cells. Store Coombs serum at refrigerator temperature because it deteriorates rapidly at room temperature. Follow the instructions that accompany the AHG reagent, especially with regard to storage and incubation time.

5-6. In addition to some of the points already mentioned, there are a number of sources of error which produce false-positive and false-negative Coombs tests. Some reasons for a false-positive reaction are:

a. Bacterial contamination of any component, e.g., cells, serum, etc.

b. Scratches in the glassware of traces of silica, which may produce the agglutination phenomenon.

c. Use of saline with a concentration in excess of 0.85 to 0.9 percent.

d. The presence of multivalent cations such as ions of copper, zinc, and iron.

Some causes of a false-negative Coombs test are:

a. Inadequate washing of the red cells.

b. Contamination of the antihuman globulin.

c. Incubation temperature above or below the optimum for the antibody being detected.

d. Inadequate incubation.

e. Absence of active serum complement.

f. Elution of the antibody by prolonged incubation.

5-7. Current studies have shown that therapy with certain drugs may result in a positive direct Coombs test. For example, one study pointed out that a significant proportion of patients taking α-methyldopa (a drug commonly used to treat hypertension) showed a positive direct Coombs test of the warm, incomplete type.[2] Another recent study pointed out that 20 percent of the patients studied who were receiving methyldopa developed a positive direct Coombs test.[3] The investigators suggested that the findings in this situation may result from drug incorporation into the red cell antigen; or alternatively, the positive test may be due to an alteration of the immune response.

5-8. **Antibody Studies.** There are three main aspects to antibody studies:

(1) Antibody detection.
(2) Antibody titering.
(3) Antibody identification.

Antibody detection is a qualitative process sometimes called antibody screening. Some of its applications are discussed in subsequent paragraphs. Antibody titering refers to the technique of preparing serial dilutions of serum to establish the concentration of particular antibodies. This is a useful procedure to follow the rise in concentration of an antibody, such as anti-D in Rh-isoimmunization, or to establish the level of anti-A and anti-B in group O blood. Antibody identification relates to steps in determining which antibody or antibodies are present in a serum.

5-9. *Antibody detection.* The qualitative presence of antibodies in serum is detected by the use of appropriate reference cells. These cells can be pooled locally or procured commercially. The use of commercial preparations is recommended because some antigens are relatively rare and will not be included unless the cell pool is taken from a fairly large number of donors. Cell pools stored at 4° C. must be prepared fresh every 2 weeks. There are several reasons for detecting antibodies: the presence of a discrepancy between cell and serum ABO grouping; crossmatch studies; preparation of low-titer, O-negative blood; prenatal workups and jaundice in a newborn; and any other routine situation in which it is desirable to screen for antibodies. Irregular blood group antibodies result from previous transfusions, parenteral injections of blood, and the isoimmunization of pregnancy.

5-10. Reference cells for antibody studies should be representative of the important antigens. In the

Rh system, antigens D, C, c, E, and e must be included. One commercially available cell reagent provides two vials of erythrocytes, one of the type R_1 (DCe) and the other type R_2 (DcE). It really doesn't matter what combination of cells is present as long as all of the antigens are there. However, this is not the only reason you should use commercial reagents rather than pooled O cells. Other antigens should also be present in the reagent cells. These include M, N, S, s, U, P, K, k, Kp^b, Js^b, Fy^b, Jk^a, Jk^b, Le^a, Le^b, Lu^b, Yt^a, Vel (Ve^a), I, Ge, and Xg^a. At least one commercial preparation that contains all of these antigens is available. Since some antigens are relatively rare, you can easily see the difficulty one might have in putting together his own reagent cells. Further, as we shall explain later, it is inadvisable from a legal standpoint to prepare your own blood bank reagents.

5-11. In a conventional procedure for antibody detection, place 2 drops of the serum to be tested in a small tube. (If your reagent test cells are separated into two vials, e.g., phenotype R_1, and R_2, you must set up two tubes.) Add 1 drop of reagent test cells to each of the tubes. Centrifuge the tube without incubation and read. Some procedures call for incubation at 37° C. for 15 minutes at this point, while other methods follow immediately with the protein phase. Add 2 drops of 22 percent bovine albumin to one of the two tubes, unless you prefer to set up the albumin and Coombs in separate tubes apart from the saline phase. After centrifugation, look for agglutination or hemolysis, and then wash the cells three times with saline. Be careful to decant the saline completely after the last wash. You can do this by standing the tube upside down on a paper towel. After washing, add 2 drops of Coombs serum to each tube, centrifuge, and reexamine for agglutination or hemolysis, indicative of a positive reaction. It is a good idea to read the Coombs test microscopically as well as macroscopically. When you read a cell suspension microscopically, always use a cover slip. This distributes the cells and permits clear observation of individual cells. A stereoscopic microscope is helpful when you are reading cell suspensions in a tube. This not only permits easy viewing but also allows comparison of one tube with another and eliminates the necessity to transfer the mixture to a slide. If the Coombs test is negative, add 2 drops of AHG-positive control cells to validate the Coombs system.

5-12. Some methods for detecting antigen-antibody reactions call for incubation at 4° C. at one point in the procedure. This is done to detect antigens that react only at low temperatures, e.g., MNS. You must distinguish agglutination at 4°C. from nonspecific cold agglutinins by warming the agglutinated cells to 37° C. If the agglutination dissipates, it is not due to a "specific" cold-reacting antibody. Controls of the patient's cells and serum should be included. With each antibody screen and crossmatch, an additional control should be used. This control tests the patient for the agglutination of his own cells, and consists of the patient's own cells and serum. This insures that the reaction obtained in the screen is not due to something in the patient's serum.

5-13. *Antibody identification.* Antibodies are identified by tests of the serum in which they occur with a panel of cells containing known antigens. The cell panels are obtained from biological suppliers, usually on a contract basis so that a fresh supply is always available. Each vial in the panel of cells contains erythrocytes with certain specified antigens. A series of tubes is set up in approximately the same manner as for the antibody screening test. A complicating feature of antibody studies is that each vial of cells contains more than one specified antigen. To identify a particular antibody, the technician must interpret his results by the process of elimination. In general, reactions in albumin are usually caused by antibodies of the Rh system. Differing reactions in different phases (saline, albumin, AGH) suggest more than one antibody. However, reactions in all phases are not necessarily an indication that there is more than one antibody present, but can many times indicate this. As an example, Rh D can react in saline, albumin, and Coombs. Varying degrees of reaction intensity also suggest multiple antibodies or dosage. By "dosage" we mean that a stronger reaction is observed with cells from a homozygous individual (e.g., cc or MM) than from a heterozygous person (Cc or MN).

5-14. Blood group specific substances A and B can be added to serum to neutralize the naturally occurring antibodies. The treated serum can then be tested for the presence of the nonneutralized immune antibodies.

5-15. Some antibodies are best detected by the use of enzymes. Treatment of red cells with enzymes may cause them to agglutinate by incomplete blood group antibodies. The effect of enzymes is, presumably, to remove ionogenic surface groups and thus reduce surface charge. Their use depends upon the situation and judgment of the technician. Enzymes are not recommended in routine compatibility testing or as a replacement for the antiglobulin test. Enzymes can also confuse the crossmatch picture, since in many instances they enhance the reaction of nonspecific agglutinins and also prevent the reaction of antibodies in the Kell, Duffy, and Kidd systems. Enzyme preparations are available commercially. However, the pH is critical, and if not optimal, antibodies may not be detected. You should use enzymes only when testing for suspected antibodies that are known to be enzyme reactive.

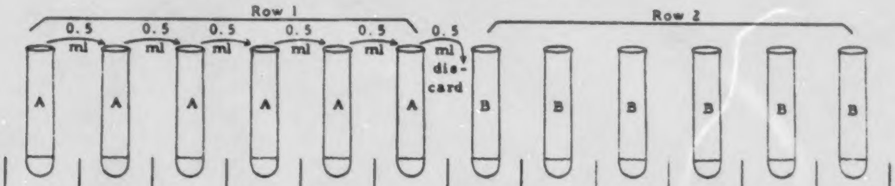
5-16. When antibodies need to be removed from serum or from the red cell, adsorption or elution procedures may be used. Adsorption separates a mixture of antibodies or removes cold autoagglutinins from serum to make serum suitable for testing. The unwanted antibody is removed by adsorption to red cells that have the corresponding antigenic determinant. This is done with selected red cells at the proper temperature. Once the antibody has been adsorbed, the serum should be tested for complete adsorption against a freshly prepared suspension of the red cells used for adsorption.

5-17. Elution is the removal of an antibody that has been adsorbed onto red cells either *in vivo* or *in vitro*. Elution is useful to demonstrate and identify antibodies on the red cells of umbilical cord blood or infant's blood in hemolytic disease of the newborn. Also, elution may be used to demonstrate and identify antibodies coating the red cells in acquired hemolytic anemia and in suspected transfusion reactions, and to separate and identify antibodies in a mixture. In all elution methods, the most critical detail is the complete removal of unadsorbed antibodies surrounding the red cells. Most eluates are prepared at 56° C.; however, when the cells are to be used for further testing, the elution should be carried out at 42° C. to 44° C., since the antigen sites may be destroyed at 56° C. The red cells should be washed at the same temperature at which the elution is performed.

5-18. *Titers.* A serial dilution of serum tells us the strength of antibodies present when this serial dilution reacts with the appropriate antigen. It is worthwhile to point out that the volume of cell suspension is not considered in calculating titers in blood banking. This is in contrast to serological titers, in which the cell suspension is taken into account in calculating the dilution factor. Table 5 illustrates a serial dilution for anti-A and anti-B. The last tube or highest serum dilution showing definite agglutination is reported as the titer of the antibody tested. If no agglutination is present in the 1:16 dilution, the titer should not be recorded. In this case, lower dilutions, i.e., 1:2, 1:4, and 1:8, should be prepared to determine the exact end point.

5-19. *Platelet agglutinins.* There is little doubt that platelet transfusions are a major advance in the treatment of certain thrombocytopenic conditions, but they are not used indiscriminately. It is known that platelets can act as strong antigens; and if antiplatelet antibodies are formed, transfusion complications may develop. As patients with secondary thrombocytopenia receive increasing numbers of platelet transfusions, the survival time of the platelets progressively decreases. This change is believed to be caused by the development of isoimmunization, which may occur as a result of exposure to group- or type-incompatible platelets. It

TABLE 5
ANTI-A AND ANTI-B SERIAL DILUTION



Clean Test Tubes	A	A	A	A	A	A	B	B	B	B	B	B
Normal Saline, ml		0.5	0.5	0.5	0.5	0.5						
Serum Being Tested	1:16 Dil.	1:16 Dil.	0.5 ml of:									
	0.5 ml	0.5 ml	1:32	1:64	1:128	1:256						
Serum Dilutions	1:16	1:32	1:64	1:128	1:256	1:512						
Diluted Serum Transferred From Row A to Row B (ml)	0.25	0.25	0.25	0.25	0.25	0.25						
Group A Cells 2 Percent Suspension	0.25	0.25	0.25	0.25	0.25	0.25						
Group B Cells 2 Percent Suspension							0.25	0.25	0.25	0.25	0.25	0.25

Centrifuge all tubes at 2000 rpm for 2 minutes. Gently dislodge the cells and observe macroscopically for clumping.

becomes desirable, on occasion, to test for the presence of platelet aggutinins. A suitable procedure *is presented in AFM 160-50. The principle of this* procedure is to detect agglutination of platelets exposed to the patient's platelet-poor plasma. A simpler test, which works with potent complement-fixing antibodies, is inhibition of the clot retraction.

5-20. *Leukocyte agglutinins.* The immunology of leukocytes is not as well understood as that of platelets and erythrocytes. It is at least an established fact that white blood cells are effective antigens. Though compatible transfusions induce leukocyte agglutinins, several factors influence their development, such as the interval between transfusions and the integrity of the antigens of the transfused leukocytes. In some instances, leukocyte isoagglutinins are demonstrated experimentally at the end of 6 to 9 weeks with the introduction of 50 ml. of whole blood weekly from a single leukemic donor with a high white cell count. In contrast, approximately 20 routine transfusions of normal blood may be necessary to produce leukocyte agglutinins (leukoagglutinins).

5-21. Erythrocytic, platelet, and leukocytic isoimmunization can develop either simultaneously or independently; but at the present time we cannot select blood donors with total compatibility for all types of cells and plasma proteins. Reactions occur in multitransfused subjects and are attributable most often to leukocyte agglutinins that develop after white cells are administered. Serious consequences may follow transfusion of whole blood or plasma from a leukocyte-immunized donor into a normal recipient.

5-22. The administration of a leukocyte-rich blood fraction to a leukocyte-immunized recipient may result, after a short period, in fever, chills, prostration, backache, nausea, and vomiting. The leukocyte, then, must be considered a potent antigen. In view of this fact, it is important that isoimmunization due to leukocytic antigens be detected and properly documented. Most laboratories use a leukocyte agglutination test to detect sensitization to previously infused leukocytes. In this test a suspension of leukocytes is mixed with the patient's serum, and agglutination occurs if the patient has been sensitized. The "mixed antiglobulin test" and the "antiglobulin consumption test" are reported to be diagnostically better tests; however, these methods have not as yet seen general application.

6. Processing Blood for Transfusion

6-1. The blood transfusion service of any hospital is one of the most important areas of activity in routine, as well as in emergency, situations. As a medical laboratory technician, you will be called upon to perform quickly and accurately in blood banking. We will describe the basic requirement of securing blood in the next chapter.

6-2. There are many clinical requirements for blood transfusions. While you should be generally familiar with the reasons for administering blood (shock, blood loss, anemia, etc.), the need is determined by a physician. Your job is to provide blood in the quantities required and perform the necessary laboratory tests to meet medical and legal specifications. At times this can be very demanding, especially when a large number of units are needed within a limited time. A properly performed crossmatch procedure can take 1 hour or more, and this is not always realized by personnel outside the blood bank laboratory. Every blood bank needs an effective system of standard operating procedures, and the system must be followed to the letter. In other words, the blood bank laboratory should be a scientific and orderly operation, not a series of crises. As in any well-organized laboratory section, there is a valid requirement for records and paperwork.

6-3. **Transfusion Records.** Within rather narrow limits, every blood transfusion service can develop its own system of keeping records. Keeping accurate and complete records is of legal importance as well as of administrative value. Documented incidents and experience have shown that most errors in blood banking are either clerical errors or misidentification of patient or donor. Records are of value in the following ways:

a. They permit orderly and effective administration of the blood transfusion service.

b. Records explain actions involving donors and patients; i.e., they provide a history of blood bank procedures pertaining to the donor, patient, and to the units of blood.

c. Accurate records reduce medical errors which could be harmful to the donor or patient.

6-4. Two of the most important records in the blood bank are DD Form 572, Blood Donor Record Card, and SF 518, Blood Transfusion. We will discuss DD Form 572 in Chapter 4 because the record card is particularly applicable to the blood donor. However, as we shall see, this is by no means the only value of DD Form 572.

6-5. *SF 518.* This form has three sections. Section I is the transfusion requisition and Section II is the transfusion record. Section III is the laboratory portion, which includes blood grouping, compatibility tests, and certification. (A copy of SF 518 is printed in foldout 2 at the back of this volume.) Section I should always be completed and the patient's identification written or printed on the 518 *before* a pilot tube of blood is drawn from the patient. Fewer errors are made if Section I is completed in full and kept with the patient's pilot tube during processing. All pilot tubes must bear a permanent label. It is a dangerous practice to work

Transfusion Number	Date	Patient's Name	Status	Ward	Physician Ordering Transfusion	PATIENT			DONOR			CROSSMATCH		Individual Performing Crossmatch	Individual Approving Crossmatch	Time Ward Notified	Time Blood Issued	Date Blood Issued	Individual Given Custody of Blood	Blood Returned?	Post-Transfusion Data
						Time Blood Drawn	ABO Group	RH Type	Blood Number	ABO Group	RH Type	Time Performed	Time Approved								

Figure 5. Sample blood bank ledger.

with unlabeled tubes. If someone brings you an unlabeled tube of blood and says, "This is from Mrs. Jones," you had better be very certain that it is indeed from the Mrs. Jones you think it is from. We do not mean to imply that in emergency situations the completion of a form has priority over all else, but there are very few emergencies that preclude the proper use of the SF 518. The few minutes needed to complete it are not usually that critical. In some cases, the physician himself prepares the requisition on SF 518, but Section I of this form is often prepared by other hospital personnel charged with the responsibility of carrying out the physician's orders. As always in your work, be aware of administrative errors. An assistant may, for example, check "Whole Blood" when the physician really wants packed cells. Though you cannot be a mindreader, experience may lead you to tactfully clarify any request that seems to be in error. A thoughtful technician respects paperwork, but he does not consider it an end in itself. The patient's welfare is his prime consideration.

6-6. Section III of SF 518 is completed as you perform compatibility tests. In some hospitals it is the practice to inform the physician when blood is ready. Ordinarily, this depends upon whether the request is routine or "Stat." The first thing you must determine when you receive a requisition is how quickly the blood is needed. What is done to complete the crossmatch and what must be done after it is completed are determined by the time element. The person who accepts a request has the responsibility for appropriate action. A request for blood is never taken lightly or set aside in deference to a coffee break. "Followup" action is mandatory.

6-7. The blood transfusion form is printed in sets of three, with carbon paper between the copies.

TABLE 6
BLOOD PRODUCTS

Whole Blood or Components	Can Be Prepared By Well Equipped USAF Clinical Lab	Clinical Uses	Storage Temperature	Expiration Time	Unit Content
Whole Blood (Fresh Drawn)	Yes	Severe hemorrhage for replacement of blood cells and coagulation factors including platelets	1° to 6°C. (Avg 4°C.)	Within 4 hours	500 ml.
Whole Blood	Yes	Severe anemia with hypovolemia. Coagulation factor deficiency (Factors VII, IX, X, or XI)	1° to 6°C. (Avg 4°C.)	21 Days	500 ml.
Packed Red Blood Cells	Yes	Exchange transfusion. Anemia without hypovolemia or with hypervolemia. Any bleeding with circulatory overload potentials. Problems of electrolyte balance who still need blood replacement.	1° to 6°C. (Avg 4°C.)	21 days in closed system or 24 hours in an open system	250 ml.
Platelet Concentrates	Yes	Severe Thrombocytopenia	None -- cannot be stored	Immediate use	30 ml.
Plasma, Fresh-Frozen Single Unit	Yes	Hemophilia and Parahemophilia. Blood volume expander in shock cases. Dehydration control. Passive immunity.	-30°C.	1 Year	200 ml.
Plasma, Stored (Pooled)	No (No longer recommended because of high risk of hepatitis)	Replacement of stable blood coagulation factors (I, II, VII, IX, X, and XI). Emergency plasma expander.	1° to 6°C.	21 Days	200 ml.

TABLE 6
BLOOD PRODUCTS (cont'd).

Whole Blood or Components	Can Be Prepared By Well-Equipped USAF Clinical Lab	Clinical Uses	Storage Temperature	Expiration Time	Unit Content
Plasma, lyophilized or aged (usually prepared from outdated whole blood or plasma units)	No (Risk of hepatitis is precluding use)	Emergency plasma expander	Below 31°C.	2 Years (aged) Indefinitely (lyophilized)	250 ml.
Plasma, platelet rich (fresh)	Yes	Severe thrombocytopenia without blood volume problems	1° to 6°C.	2 Hours	250 ml.
Anti-Hemophilic Factor Cryoprecipitate Concentrate (Fresh)	Yes	Replacement of Factor VIII	-30°C.	(Under investigation)	25 ml.
Albumin (usually fractionated from outdated whole blood or plasma units)	No	Temporary replacement for cirrhotic patient albumin. Restore plasma volume. Used to bind bilirubin in conjunction with exchange transfusion; shock	1° to 6°C.	5 Years	Various sizes available
Fibrinogen (desiccated from outdated whole blood or plasma units)	No	Hypofibrinogenemia	1° to 6°C.	Indefinitely	2 gram vials for reconstituting
Immune Globulin (intramuscular preparation from hyperimmunized patients)	No	Passive protection by providing high titer antibodies	1° to 6°C.	3 Years	0.05g/Kg doses
Leukocytes (Fresh -- usually taken from buffy coat of packed cells from specific donors)	Yes	After suppressive therapy. In severe infections.	1° to 6°C.	6 Hours	25 ml.

When you send a unit of blood to the ward, to surgery, or wherever it is needed, you keep one copy of the SF 518 and forward two. Mark the second copy "Return to the Laboratory." Keep the third copy as a suspense copy in the laboratory. When blood is given, the physician signs the SF 518, and the copy you have marked returns to the laboratory. At this time you file it and destroy your suspense copy. Each day you check your suspense copies. If a record has not been cleared (if the completed second copy has not been returned), you may have to trace the unit of blood. Blood that is not administered is sometimes kept in refrigerators in surgery or on the ward. This is not permitted unless such refrigerators meet all the requirements prescribed by the American Association of Blood Banks for the storage of blood. We will discuss most of these requirements in Chapter 4. Moreover, some blood bank directors prefer as a matter of safety to keep crossmatched blood under blood bank control and to release units only as they are needed for transfusion.

6-8. *Blood bank ledger*. Another transfusion service record, in addition to the two forms already mentioned, is a blood bank ledger. There is no standard form for this purpose, but any notebook will suffice. Columns are headed with all pertinent information, as illustrated in figure 5. A record book of this type consolidates all of the operational information necessary to control the issuing of blood. Take a few minutes to study figure 5.

6-9. *Local requirements.* AFM 160-50, *Laboratory Procedures, Blood Banking and Immunohematology,* recommends keeping a transfusion record on all patients. It would appear that a complete file of SF 518s could be substituted to meet the requirement of a transfusion record. Accreditation requirements and the standards of the American Association of Blood Banks provide for a transfusion committee. Hence, some system must permit the orderly review of transfusion records on all patients. There is no standard system. Local commanders usually establish policy based upon recommendations of the accrediting agency. The pathologist or clinical laboratory officer is usually a member of the transfusion committee and establishes guidelines for blood bank transfusion records maintenance. Recipients' inpatient records are available, and all SF 518s are ordinarily reviewed by the committee.

6-10. **Blood Components.** Blood that is collected from a donor is generally transfused as whole blood, but there is a trend toward greater therapeutic use of blood components. Blood component therapy refers to separating the elements of the blood and transfusing these elements to patients as needed. For example, many patients need only the red blood cells; in such instances, the physician may specify transfusion with packed erythrocytes. The plasma can then be processed and fractionated for other purposes. Refer to table 6, and note the many items that can be obtained from a unit of while blood. Because blood is such a precious commodity, it should not be wasted. Researchers are attempting to discover ways of freezing whole blood as well as preserving blood components that will insure stability and long-range storage. Fresh frozen plasma must be stored at minus 20° C. for adequate preservation.

6-11. Platelet transfusions are part of a blood bank service. Platelet deficiency occurs in thrombocytopenia and in excessive platelet consumption associated with carcinomatosis, hepatic cirrhosis, acute leukemia, and other diseases.[4] In addition, there are several drugs that induce an immunologic thrombocytopenia, e.g., digoxin and the thiazides. Freshly collected blood is an excellent source of platelets. In some cases, blood from polycythemic patients or patients with thrombocytosis is used; however, the use of such blood calls for a medical judgment. Platelet-rich plasma is often used with acid-citrate-dextrose (ACD) as the anticoagulant. Adding excess ACD prevents agglutination of platelets when platelet-rich plasma is used. You can find discussions of platelet transfusions in current medical literature. One recent study has shown, for example, that a profound decrease in platelets is experienced in cases of ABO incompatibility.[5] The same study points out that Rh incompatibility did not seem to accelerate the destruction of platelets.

6-12. In addition to the components listed in Table 6, several other preparations and products are used. Such combinations as leukocyte-poor whole blood, platelet-poor packed cells, and cryoprecipitate concentrates are well known in the blood transfusion service. A clinical laboratory working in close cooperation with the physicians can prepare some of the needed transfusion products for optimal patient benefit. Specially prepared components can be procured through supply channels or commercially and stocked by the blood transfusion service. Many laboratories obtain blood components through a plasma exchange plan offered by pharmaceutical firms. This arrangement is worthwhile because most Air Force hospitals are not equipped to fractionate blood beyond the separation of cells and plasma. When a blood unit has been entered with a transfer pack or in any way, it is considered an open system, and a 24-hour maximum dating period must be observed.

6-13. **The Crossmatch.** Sometimes referred to as a compatibility test, the crossmatch is a test between the blood of a person who is to receive a transfusion and the blood of a donor. The test is performed to reveal a possible incompatibility between the donor's blood and the recipient's blood. Agglutination or hemolysis on the major side (donor's cells and patient's serum) or on the minor side (patient's cells and donor's serum) is considered evidence of incompatibility. If the blood is incompatible, it is not administered to the patient. In place of the minor crossmatch, a broad spectrum, donor antibody screening test is sometimes performed. In any case, the donor's serum must be adequately tested for immune antibodies. By "adequately" we mean that the test should be carried out in saline, albumin, and AHG at room temperature, at 37° C., and at lower temperatures for both specific and nonspecific cold agglutinins. Most immunohematologists suggest using the minor crossmatch in addition to antibody studies.

6-14. The primary purpose of a crossmatch is to prevent a transfusion reaction. Procedures vary somewhat as methods improve. Therefore, no one method can be learned as the ultimate in technique. But it is of the greatest importance that you intelligently apply the prescribed and accepted method of the facility in which you are working. Never attempt to modify a procedure or take shortcuts that are not authorized. To do so would be to assume unnecessary responsibility and perhaps even jeopardize the life of the patient.

6-15. A complete crossmatch includes saline, high-protein, and antihuman globulin test systems. Enzymes may also be included, but should never be considered a substitute for one of the other phases of a crossmatch. A synthetic substitute for albumin is available commercially, but this product has not

TABLE 7
ANTIBODY DETECTION IN VARIOUS PHASES OF A CROSSMATCH PROCEDURE

CROSSMATCH PHASE	PRINCIPAL ANTIBODIES DETECTED
SALINE MEDIUM	
Room Temp.	ABO incompatibilities Cold agglutinins, P, MNS Lewis, Lu, Wright.
At 37°C	Most Rh-Hr, Lewis Differentiates cold agglutination.
HIGH PROTEIN	Rh-Hr
ANTI-HUMAN GLOBULIN (AHG)	Most Rh-Hr Duffy, Kidd, Kell, and certain other antibodies are detected only by AHG.

been thoroughly researched by the Air Force to determine its advantages or possible disadvantages. Sufficient albumin to arrive at a final concentration in the reaction mixture of 15 to 20 percent must be used. This usually requires 3 drops of 30-percent bovine albumin. It is also recommended that the high-protein treatment precede the Coombs test because there are albumin-enhanced AHG-reactive antibodies. Serum-cell mixtures are incubated at room temperature and 37° C. Antigen-antibody reactions that take place in these media are thus detected, as illustrated in table 7. You should realize that a crossmatch does not detect all ABO grouping errors, e.g., erros due to weakly reacting subgroups. Neither does the crossmatch detect errors in Rh typing unless the serum contains an Rh-antibody. (Remember that Rh antibodies are not "naturally occurring.") Moreover, a compatible crossmatch is no assurance that isoimmunization will not occur.

6-16. Your job as a blood bank technician is to provide the physician with a blood product that will be beneficial and safe for a patient. The test tubes represent the patient's circulatory system. If an incompatibility is detected in your test tube crossmatch, the dire consequences of a transfusion reaction taking place in the patient are predictable. Extreme caution and demanding concentration are essential requirements in the blood bank. Before you provide a unit of blood for use, you must check it for contamination and hemolysis visually. Inspect plastic bags for leaks or defects. Check all identifying numbers and log the blood out properly. Further patient identification should be established before you issue a unit of blood for transfusion. This may be done by issuing blood to legally responsible hospital staff members only and requiring that they submit a request slip containing the intended recipient's name, date, ward, and register number.

6-17. AFM 168-4, *Administration of Medical Activities,* requires that blood earmarked for a patient will be held in a laboratory for 48 hours after being typed and crossmatched. It is then made available for retyping and recrossmatching for another patient. Of course, in certain specified cases the physician may require that blood be held for a longer period. Whenever blood is released, the physician should be advised of this action because, strictly speaking, only the physician who orders blood has the authority to release it. Never overcommit a particular unit of blood. To crossmatch the same unit of blood for more than one patient simultaneously is to ask for trouble.

6-18. **The Emergency Crossmatch.** If a surgical patient hemorrhages or suffers any other sudden blood loss, the blood banker is called upon to furnish replacement blood as soon as possible. The physician may demand uncrossmatched blood on the basis of his clinical judgment and his evaluation of the urgency of the situation. It is part of the physician's job to accept full responsibility for this action.

6-19. AFM 168-4 states "It is imperative that

TABLE 8
EMERGENCY CROSSMATCH GUIDE

60 minutes	Full crossmatch-saline albumin, Coombs
30 minutes	Release blood after albumin phase. Continue to complete crossmatch.
15 minutes	Release blood after saline and albumin phase. Continue to completion.
5 minutes or less	Give ABO group and Rh type-specific blood uncrossmatched. Begin complete crossmatch. If necessary, give low-titer O negative blood.

final typing and crossmatching be accomplished prior to all transfusions. The typing and crossmatching should be doublechecked and countersigned by a person familiar and current with the detailed techniques and procedures being utilized."

6-20. When time does not allow crossmatching, ask the physician to sign a release form acknowledging his responsibility and accepting the risks in giving uncrossmatched blood. This action helps protect you legally from personal liability in this situation. Obtain a sample of blood from the patient and from the pilot tube before the unit leaves the bank and begin a routine crossmatch. At the first sign of incompatibility, notify the physician. Do this through the officer in charge of the blood transfusion service. If the samples are compatible, complete the necessary SF 518. Emergency crossmatching techniques can be used to prepare blood when time is short. Table 8 is a theoretical, descriptive guide for handling emergency situations; but if local policies direct otherwise, they should be followed.

6-21. If blood is released without the complete crossmatch, appropriate records must reflect this action. Whenever a patient is transfused, there is some risk, either immediately as a transfusion reaction or as subsequent sensitization. The omission of crossmatch safeguards increases that risk. Deciding when to transfuse blood is entirely the responsibility of the physician, and the blood banker is thereby obligated to make the blood available. You must enter the information in blood bank records and immediately notify the chief of the blood transfusion service whenever you release blood without a complete crossmatch.

6-22. **Miscellaneous Problems.** In addition to the points previously covered, there is a variety of related problems. We will mention some of the most common difficulties encountered in a blood transfusion service.

6-23. *Additives.* Nothing should be added to a unit of blood in the blood bank by blood bank personnel. If a biological (e.g., Witebsky group-specific substance) is desired by the physician, and he wishes to add this or other substances (e.g., insulin) to the blood, the addition can be made by the medical staff attending the patient.

6-24. *Use of the universal donor.* The use of O negative as universal donor blood is *not* as desirable as using the patient's own blood type. If you do crossmatch O negative blood with an A or B patient, would you expect compatibility on the minor side?

6-25. *Incompatible crossmatch.* Some of the causes of an incompatible crossmatch and suggested followup action are given in table 9. From a practical standpoint, it is often simpler to select a new unit of blood for crossmatch than to undertake detailed studies; but be sure you have not made a common error like mistyping.

6-26. *Multiple transfusions.* If a patient receives

TABLE 9
CROSSMATCH INCOMPATIBILITY PROBLEMS

Observed Incompatibility	Some Possible Causes	Action Required
I. Saline or serum room temperature	(1) ABO error	(1) Front type and Back type donor and recipient
	(2) Cold agglutinin	(2) Allow recipient blood to clot in refrigerator (b) Agglutination dissipates at 30-37 degrees C.
	(3) Irregular antibody	(3) Identify antibodies with reagent cells (e.g. "Panocell")
II. Saline, serum or high protein at room temperature.	(1) Irregular antibody	(1) Same as I (3)
	(2) Autoagglutinin	(2) Auto-adsorption; see I (2)
	(3) Rouleaux	(3) Add saline
III. AHG or Enzyme	(1) Irregular antibody	(1) Same as I (3)
	(2) Autoagglutinin	(2) Auto-adsorption; see I (2)
	(3) Positive direct Coombs	(3) Check direct Coombs on donor or recipient as indicated
IV. Any phase	Technical errors	Investigate following: Dirty glassware; bacterial contamination; chemical contamination; fibrin clots; over-centrifugation or centrifuge vibration.

several units of blood, a new sample must be obtained from the patient each day for crossmatch. Interdonor crossmatches are not necessary.

6-27. *Antibody identification.* If a patient possesses an antibody that consistently results in incompatibility, this antibody must be identified. Panels of cells containing known antigens are available for this purpose. When you have identified the antibody, you must use specific antisera to select donor blood that does not contain the corresponding antigen.

6-28. *Use of fresh blood.* It is estimated that the post-transfusion survival of red blood cells decreases by less than 1 percent for each day of pretransfusion storage. Platelets are far more vulnerable to storage, and it is therefore desirable that platelet concentrates be no more than 6 hours old. Leukocytes have a relatively short lifespan in stored blood, and the number of leukocytes contributed by a unit of blood is relatively small. For this reason, correction of leukopenia by the use of whole blood is virtually impossible. Relatively few cases require the use of freshly drawn blood. One such instance is, as suggested, the need for platelets.

6-29. *Transfusion complications.* Patients who receive blood may suffer a number of possible reactions. These include pyrogenic reactions, allergic reactions, embolism, and hemolytic reactions. It is also possible for a recipient to be infected with bacteria or viruses. Though none of these conditions is necessarily probable, their possibility requires a certain amount of knowledge on your part. Let us briefly consider each reaction.

6-30. There is very little you can do to prevent or follow up a pyrogenic reaction. If the patient develops a fever from chemical substances in the blood-anticoagulant mixture without associated complications, this is termed "a pyrogenic reaction." Pyrogens occur in the anticoagulant diluent as products of bacterial contamination. In most instances, the problem is to distinguish a pyrogenic reaction from a more severe transfusion reaction. As a rule, pyrogenic reactions are not fatal. An allergic reaction is a response to practically any substance in the blood to which the recipient is sensitive. Its severity varies from itching and hives to respiratory collapse. Again, this is a systemic response over which the blood bank has very little control, provided the response is not caused by contaminated blood. An allergic response is usually considered separate from incompatibility reactions in which hemolysis or agglutination of the erythrocytes is involved.

6-31. An embolism is the obstruction of a blood vessel by a clot, foreign particles, or an air bubble. Proper mixing of the blood with anticoagulant during collection prevents clots from forming in the blood bag. Ordinarily, any clots present are removed by the filter in the blood administration set. When you collect blood, keep air from entering the bag by keeping the metal ball valve in place until the needle is in the vein. When blood is transfused under positive pressure, air in the bag may be forced into the recipient.

6-32. Another situation that can be critical is a hemolytic transfusion reaction. Hemolytic reactions are caused by intravascular hemolysis of either the recipient's or the donor's erythrocytes, usually the latter. The severity of a hemolytic reaction depends upon the degree of incompatibility, the amount of blood administered, the rate of administration, and the physical condition of the patient. Clinical symptoms are not of direct concern to the laboratory, except that hemolytic transfusion reactions are serious medical outcomes of administering incompatible blood. You should realize that most errors in blood banking are the clerical errors of mislabeling and misidentification. You can minimize hemolytic reactions by keeping accurate records, by using properly stored blood, and by practicing the principles of blood banking that are discussed in this CDC. Never heat blood in water baths or in any other way contribute to hemolysis of the erythrocytes. Hemolysis can be caused by rough handling of the blood bags, exposure to extremes in temperature, and delayed refrigeration. Occasionally blood will hemolyze because of unusual red cell fragility, especially after the blood has been stored for several hours or days. The hospital staff should be educated by in-hospital programs to acquaint them with ways to reduce the incidence of reactions. For example, slow administration of the first 50 ml. of blood under close observation can detect untoward reactions before the consequences become serious. Finally, you should be prepared to follow up reported transfusion reactions. Instructions to the physician in cases of transfusion reaction are listed on SF 518 (see foldout 2). Local policies may give further instructions.

6-33. Minimum followup action to transfusion difficulties includes a complete urinalysis of the recipient; reaccomplishment of all typing and crossmatching procedures; reexamination of all identification data, including labels, record books, and the like; checking to be certain that pilot tubes correspond to their respective blood units; and making certain that the recipient was the patient for whom the blood was intended. Laboratory supervisory personnel must be personally involved in all followup action. The supervisor of the blood bank should establish a written procedure for the laboratory technician to follow in investigating a transfusion reaction. A local investigation form could be developed with instructions for transfusion reaction investigation. In some cases it may be desirable to call upon a consultant laboratory to substantiate your conclusions. Special antibody studies are frequently in order, and chemistry determinations, e.g., the bilirubin test, may be appropriate. When blood is first crossmatched, all pilot tubes should be labeled to include identification, time, and date. These tubes are saved until the SF 518 is returned to the laboratory indicating a successful transfusion. In the case of the donor, "identification" means unit number. A patient's pilot tube should be identified by name and registry number. Special care must be taken not to "clutter" the blood bank with old tubes that might be mistakenly used or mixed up with blood for another patient, or even confused with subsequent samples from the same patient.

6-34. The infections most frequently transmitted by blood transfusion are hepatitis, malaria, and bacterial infections, although other possibilities also exist. A carefully prepared donor history and a VDRL or suitable substitute reduces the danger of transmitting hepatitis. Daily examination of blood banks under a light or with a flashlight for unusual cloudiness, pellicles, or opaque aggregates will reveal bacterial contamination. In some cases, you will also be able to detect breaks in the blood bags, which lead to bacterial contamination. Hemolysis and icteric plasma will also be apparent. We will discuss collection and storage of blood in the following chapter.

6-35. *Blood bank reagents.* Twenty years ago many laboratories were preparing their own blood bank reagents. Today very few clinical transfusion services make any of their own reagents. While the potential for preparing almost any blood bank reagent does exist in many laboratories, medicolegal problems dictate the use of products from reputable sources licensed by the Federal Government. American liability laws depend heavily upon community medical practices. If you should be so "economical" as to produce your own blood bank reagents, a transfusion reaction or other complication could have serious legal implications. If the person who suffered the reaction can show that every other blood bank in the geographic area used Brand X reagents, you have a good chance of incurring legal liability for the injury. The Federal Government has designated the National Institutes of Health (NIH) to monitor and license all blood bank reagents. NIH approves or disapproves all commercial blood bank products and acts as a safeguard on commercial blood bank reagents for the United States. To meet the high standards of the Federal Government, commercial companies employ rigid quality control measures. Quality control and legal discretion go hand in hand to promote high standards in clinical transfusion services. Be certain that attention is given to checking the potency of your reagents, their proper storage, and your awareness of bacterial contamination.

6-36. **Hepatitis-Associated Antigen (HAA).** Until a few years ago no agent or substance thought to cause serum hepatitis was known. Blumberg in 1964 discovered an unusual antigen in the blood of an Australian aborigine, and later studies linked this antigen with the severe serum hepatitis that frequently occurred after a patient had received a transfusion of blood or blood components. Subsequent studies by means of electron microscopy have shown that the bloods of persons capable of transmitting hepatitis contain hepatitis-associated antigen (HAA), a virus-like particle approximately 20 mμ in diameter. This particle when transmitted can give rise to serum (long-term) hepatitis. The particle has not been found in cases of viral (short-term) hepatitis.

6-37. Hepatitis-associated antigen or HAA, as it is commonly called, is known by various names. Included among these are SH antigen and Australian antigen. Federal law presently requires that all blood for transfusion purposes be checked by an FDA-approved method (currently counter immunoelectrophoresis or CEP). A recently introduced method employing radioimmunoassay techniques appears to be more sensitive in detecting HAA than methods developed so far. Methodology for detecting HAA is summarized below:

a. Gel Diffusion. This is a modified Ouchterlony double diffusion method employing purified agar. This is the simplest procedure; however, it is not the most sensitive method. The main disadvantage in this procedure is that an optimum ratio of antigen and antibody necessary for maximum precipitation is difficult to obtain.

b. Hemagglutination Inhibition. This test method has been used; however, it is somewhat lacking in specificity and sensitivity.

c. Complement-Fixation. Standard CF methods have been successfully used and are more sensitive than gel diffusion and hemagglutination inhibition methods but are more complex and difficult to perform.

d. Inhibition Crossover Electrophoresis (ICEP). This electrophoretic method has been used. It involves neutralizing HAA in a test serum with standard antibody, and the product of this reaction is electrophoresed on an agarose gel film against a standard antigen (HAA) reagent. A lack of precipitation lines indicates that the standard antibody solution added to the test serum reacted and was neutralized.

e. Counter Immunoelectrophoresis (CEP).[6] This method is the one presently required by FDA regulations. Multitest agarose agar plates containing a series of opposing wells are employed. Serum to be tested for HAA is placed in a cathodic well, and standard anti-HAA is placed in an opposing anodic well. Electrophoresis is accomplished at room temperature, and the presence of HAA is indicated by a line of precipitation between the opposing wells. Anti-HAA and antibodies can also be detected using CEP. In this procedure a standard antigen reagent is put in the well on the cathode side, and the test serum is put in the opposing anodic well.

f. Radioimmunoassay (RIA).[7] This is the most recently introduced, commercially available method. It is reported to be more than 100 times as effective as the presently required method (CEP) in detecting HAA and is expected to become the FDA-approved method. In this procedure, serum to be tested for HAA is put into a tube containing a film of gel impregnated with anti-HAA. During an incubation period, HAA in the test serum attaches to the anti-HAA in the gel film. Contents of the tube are then decanted, and the HAA-antibody complex remaining in the tube is washed and rinsed several times. A labeled (I^{125}) anti-HAA is then added to the tube. The labeled anti-HAA attaches to the HAA, creating a sandwich effect. The HAA is bound on one side by anti-HAA in the gel film and on the other by the I^{125} labeled anti-HAA. Washing and rinsing remove excess labeled antibody. The amount of labeled antibody in the tube is determined in an autogamma spectrophotometer, and this amount is proportional to the amount of HAA in the tube. The method is very sensitive and is said to detect HAA levels undetectable by other methods. The main disadvantage is the complexity of the procedure and the expensiveness of the materials and equipment.

g. Latex Agglutination. Workers in England report that they were able to adapt the latex agglutination technique to the detection of HAA. They report that it is as sensitive as gel diffusion and CEP.

Footnotes

[1] Charles Singer and E. A. Underwood, *A Short History of Medicine*, New York: Oxford University Press, 1962.

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[3] A. Breckenridge *et al.*, "Positive Direct Coombs Tests and Anti-Nuclear Factor in Patients Treated with Methyldopa," *Lancet*, Vol. 2, No. 7529 (1967), pp. 1265-1268.

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[6] H. J. Alter, P. V. Holland, and R. H. Purcell, "Counter Electrophoresis for Detection of Hepatitis-Associated Antigen: Methodology and Comparison with Gel Diffusion and Complement Fixation," *J. Lab. and Clin. Med.*, Vol. 77, No. 6 (1971), pp. 1000-1010.

[7] J. H. Lewis and J. E. Coram, "Australian Antigen Detection. Comparison of Results Obtained with Five CEP and One RIA Test Systems," *Transfusion*, Vol. 12, No. 5 (1972), pp. 301-305.

CHAPTER 4

The Blood Donor Center

"WE SWEAT BLOOD" is an advertising slogan occasionally used by a leading manufacturer of blood banking supplies. This might well be the motto of most blood bank technicians in the Air Force. To only the most fortunate workers is a bountiful supply of blood for transfusion delivered to the laboratory, neatly lableled and ready for use.

2. In recent years, requirements for shipping blood to U.S. combat forces overseas have placed an even greater demand on military blood banks. They must not only supply their own day-to-day needs but must also support the military blood program in other geographic areas as directed. Blood donor center operation, and the procurement of blood routinely and during national emergencies, will be discussed in this chapter.

3. Since the establishment of the first blood bank in the United States at Cook County Hospital in Chicago in 1937, blood banking in the United States has become a vital institution as well as big business. As members of the Armed Forces, we are expected to meet our needs by cooperation with community blood banks and organized agencies, such as the American Association of Blood Banks and the American Red Cross, and by our own efforts. Sometimes a combination of these sources provides the simplest solution.

4. In the final analysis, blood must be provided, and there is only one original source, the blood donor. To complicate matters even further, whole blood is suitable for transfusion only within 21 days after it is drawn. Only the alert and resourceful blood banker can continue to function under such imposing restrictions, and, as a matter of fact, blood banks do frequently find themselves in a precarious position with respect to the availability of blood.

7. Collection of Blood

7-1. As a blood bank technician, you will be responsible for seeing that an adequate blood supply for transfusion is available. Since the only source of human blood is a human donor, the blood donor must be well treated and protected. You must apply your technical training to care for the donor and encourage him to return to donate again. By proper donor-technician relationships, the clinical transfusion service supply system can be maintained. Just as a rule in medicine is "do not harm the patient," the rule in blood banking is "do not harm the donor *or* the patient." In this section we will describe donor eligibility and accepted techniques of drawing blood for transfusion.

7-2. **Donor Criteria.** You obviously cannot draw blood from just anyone who walks through the door of your laboratory. On the other hand, it is not practical to provide a complete physical examination for every blood donor. Rather, the blood banker must rely on a statement of medical history from the prospective donor and a few simple medical factors (usually temperature, pulse, blood pressure, and hemoglobin). The findings of the examination and a brief medical history are recorded on DD Form 572, Donor Record Card. A facsimile of DD Form 572 is included in foldout 2 of this volume. Before proceeding, turn to the foldout 2 and fill out DD Form 572 as if you were the donor. Also, enter what you consider to be normal data for your weight, temperature, pulse, blood pressure, and hemoglobin. Then, review your entries after you have read this section and determine if the information you have recorded on DD Form 572 qualifies you for donation.

7-3. DD Form 572 is an important blood banking record. It lists pertinent facts about the donor's health, which are useful in protecting the donor and the patient; but remember, a record of facts is by itself of limited value. It is your responsibility to interpret this information. Have you assured yourself that the donor is healthy before taking his blood? If a donor suffers a reaction, the DD Form 572 can protect you. On the other hand, if you disregarded low hemoglobin, high blood pressure, or other disqualifying factors, the blood donor record card can be incriminating indeed!

7-4. Here are some additional ways in which the blood donor record card is of value:

a. DD Form 572 tells you something about a unit of blood. It is, in a sense, the medical history of the unit.

b. The donor record card permits followup. It tells you how often the donor gave blood at your facility. If a patient develops hepatitis or any other transfusion-related condition, necessary followup action can be taken. This may include treating the donor or removing his name from the donor list. When information concerning the health of a donor requires, it is your responsibility to make this known to the military health section or other appropriate unit of the hospital. When in doubt, consult your supervisor.

c. The form tells you who collected the blood. This can be of some value, especially if the unit becomes contaminated or in some way unsatisfactory.

d. DD Form 572 helps identify the unit because it bears an accession number, which also appears on the unit.

7-5. Let us now turn our attention to specific eligibility criteria. A medical officer should approve all blood donors, and sign DD Form 572 in the space provided. There are instances in which the criteria cited here vary from the requirements given in AFM 160-50 (Nov 1966). For example, many workers argue against drawing blood from a donor with a history of malaria or polycythemia. Authoritative references on donor criteria are available in most hospital libraries. One such authoritative text is published by the American Association of Blood Banks, 30 North Michigan Avenue, Chicago, Ill., 60602. It is entitled *Technical Methods and Procedures of the American Association of Blood Banks.*

7-6. *Age*. The usual permissible age range for blood donors is 21 to 60 years. Donors who are less than 21 yerars of age are accepted if (1) they are members of the Armed Forces over 17 years of age, (2) they are civilian minors for whom signed consent is given by the parent or legal guardian, or (3) they are married and over 17 years of age.

7-7. *Previous donation*. A period of not less than 8 weeks should have elapsed since approximately 450 ml. of blood was withdrawn from the individual. No more than five donations are allowable within a 12-month period. In disaster situations or during periods of national emergency, exceptions are granted under the provisions of AFM 160-16, *Operational Procedures for Military Blood Donor Centers* (Oct 1959). In normal circumstances the time limitation should be followed.

7-8. *Nourishment*. Blood to be used for transfusion must be collected from donors who have refrained from eating fatty foods for at least 3 hours. It is preferable to permit donors to eat nonfatty meals rather than to enforce a period of food abstinence. Too long a period of strict fasting lowers the blood sugar and increases the incidence of donor reaction.

7-9. *Disease*. A person who has once had serum hepatitis should not be permitted to donate blood. Presently accepted blood banking practices hold that blood from a person with a history of viral hepatitis connot be used, even for fractionation. History of malaria is cause for permanent rejection. A history of syphilis is also cause for rejection unless the donor can prove that he was adequately treated. Further, he must have a nonreactive VDRL.

7-10. Prospective donors with a history of undulant fever (Brucellosis) may be accepted if they have had no attack in the preceding 2 years. All individuals who have had active clinical tuberculosis are disqualified. Persons with upper respiratory disease, such as colds, fever, flu, persistent cough, sore throat, and pain in the chest, must be rejected until at least 1 week after active symptoms have subsided. It is usually necessary to consult a physician to be sure the donor's symptoms do not indicate some underlying disorder.

7-11. Persons with a history of infectious mononucleosis are excluded until they have completely recovered. Cases of skin infection should be referred to a physician, as should people with minor allergic manifestation, such as hayfever, hives, and skin rash. A history of repeated attacks of asthma, even if inactive at the time of examination, is disqualifying.

7-12. Persons showing a history of convulsive seizures and frequent fainting (except in infancy) are not acceptable. Individuals who are known to have heart disease, such as rheumatic fever or cornary artery disease, are not acceptable. Nonacceptability also applies to persons who have hypertension with a systolic pressure above 200, diastolic above 100, or hypotension with a systolic pressure below 100 and a diastolic pressure below 50. Also, persons with a history of shortness of breath, swelling of the feet and ankles, angina, and pain in the chest must be rejected. Individuals with diabetes who require drug therapy are also disqualified. An abnormal bleeding tendency or any active blood disease is cause for rejection. Persons with a history of either active or chronic liver or kidney disease should be rejected.

7-13. Particular note should be made of the presence or repeated occurrence of minor respiratory problems. No arbitrary limit can be set regarding the interval after which the donor becomes eligible for donating blood, because of the variability, severity, and duration of problems such as sinusitis, allergy, or hay fever. Donors with chronic sinusitis and hay fever are acceptable if they are not in the acute stage and are otherwise in good health. Detailed information should be obtained from donors who give a recent history of septic sore throat, and they should be accepted only after consultation with a physician.

7-14. Current philosophy toward immunizations is that inoculations of vaccines for polio, influenza, thyphoid, measles, typhus, tetanus toxoid, cholera, and diptheria toxoid are disqualifying for 1 week following inoculation or until there is no evidence of local reaction, whichever is longer. There is no restriction for donors who have received oral polio vaccine. Injections that are disqualifying for 2 weeks include tetanus antitoxin and vaccines of live virus, such as yellow fever and smallpox. Immunizations to prevent rabies are disqualifying for a period of 1 year.

7-15. *Hemoglobin*. It is very important that a donor's hemoglobin be 12.5 gm. percent or higher for a female and at least 13.5 gm. percent for males. Equivalent acceptable hematocrit levels are 37 vol. percent and 40 vol. percent, respectively. It is not the proper sequence of events to draw the unit of blood first and then check the hemoglobin level. Considerable harm can be caused by drawing blood from an anemic donor. Further, never draw more than 480 ml. of blood (450 ml. into the bag plus 30 ml. into the pilot tubes). The donor's health is a primary consideration. In addition, blood that is drawn for transfusion purposes must be the best available.

7-16. *Pregnancy*. Pregnant patients are not permitted to donate. A donor who has been pregnant is disqualified for donation until 6 months after the pregnancy has been terminated.

7-17. *Medication*. Any person who has taken medications, including oral contraceptives, weight reduction pills, or other drugs, should be referred to a physician. If possible, the physician consulted should be the one who prescribed the medication in question.

7-18. *Other criteria*. Individuals under the care of a physician for any reason should be cleared medically before donation. Mental patients are not recommended as donors because medical history or consent statements made by patients under psychiatric treatment are voidable in a court of law. Prospective donors who have received a blood transfusion or had major surgery are excluded from giving blood for 6 months. Dental surgery excludes a donor for 72 hours.

7-19. *Flying personnel*. AFR 160-26 *Air Force Blood Program* (13 Oct 72) states: "Aircrews of high performance aircraft and persons occupying cockpit positions in an on-call status to perform essential flight duties will not donate blood."

7-20. Donors must weigh at least 110 pounds. The donor's oral temperature should be between 97.6° F. and 99.6° F. As previously mentioned, his blood pressure should be between 100/50 and 200/100. Donors with an abnormal variation between their systolic and diastolic blood pressures, e.g., 200/50, are referred to a physician for approval. The pulse rate must be between 50 and 110 beats per minute.

7-21. *Collection Techniques*. Standard blood-collecting equipment consists of a plastic bag with an integral plastic tube attached, as shown in figure 6. Units are available for collecting 500, 450, or 250 ml. of blood in 75, 67.5 and 37.5 ml. of ACD USP Formula A anticoagulant, respectively. Plasma, cells, or a portion of the whole blood may be transferred from the plastic bag to a transfer pack, which is illustrated in figure 7. Cells can be

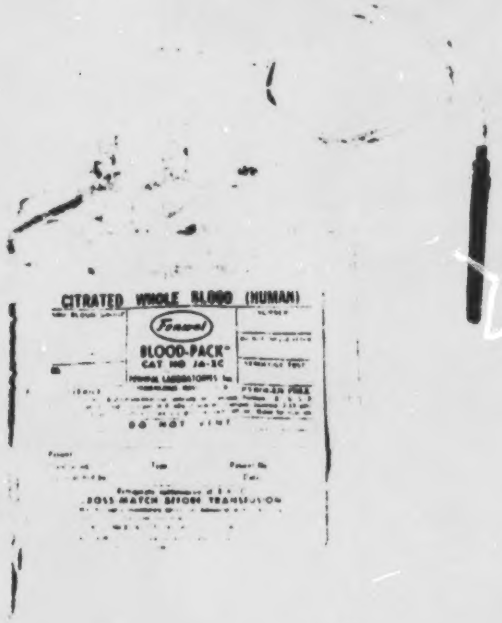

Figure 6. Blood collecting bag.

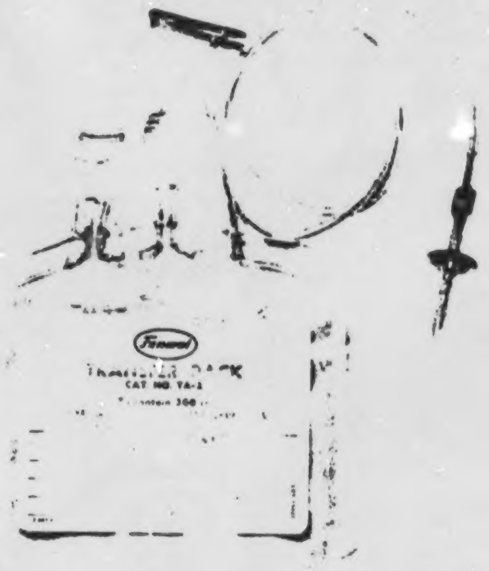

Figure 7. Transfer pack.

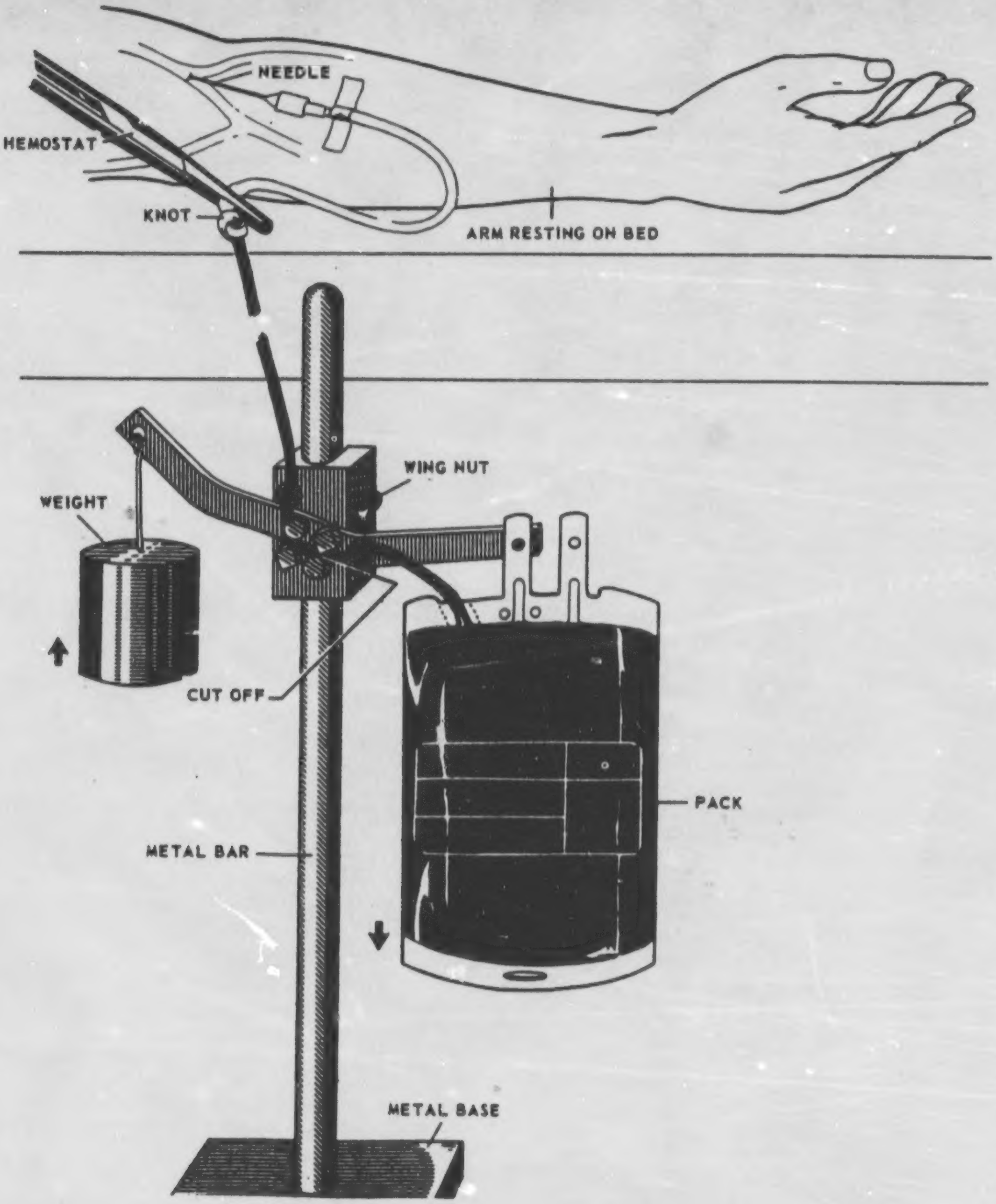

Figure 8. Blood collecting balance.

separated from plasma by centrifugation or gravity. A large book can serve as a compress if a plasma extractor is not available.

7-22. The plastic bag is composed of an inert polyvinyl plastic, which is less damaging to thrombocytes and cells than a glass surface. The needle is a large gauge, stainless steel phlebotomy needle firmly attached to the plastic tubing. Once the needle cover is removed, the needle should be used at once because it cannot be considered sterile if the cover is replaced. The donor tube is imprinted with a repeating series of numbers so that it can be sealed into identified segments for laboratory use. The tube also contains ACD. A stainless steel bead is positioned at the juncture of the tube and the bag. The bead acts as a valve to keep in the anticoagulant.

7-23. When you are setting up the container for blood collection, you may use either a spring scale or a weight balance. The collecting balance, shown in figure 8. has the advantage of providing an automatic shutoff if the donor tube is strung through as shown. Occasionally the donor tube pinches off if it is not placed correctly through the cutoff arm. This restricts the free flow of blood. Unless a vacuum chamber is used, the bag should be hung well below the level of the donor's arm. Be sure that the label on the bag matches the donor number on DD Form 572 (Blood Donor Record Card) and on any additional pilot tube that may be used.

7-24. Before you position the donor on the table or in the donor chair, review his record card and be sure that he is eligible to give blood. Then you should examine both of the donor's arms to select the arm with the best vein. Sometimes you must apply a tourniquet or blood pressure cuff before you can make this determination; but do select the better arm before proceeding with a skin prep. When you have decided which vein you wish to use, position the donor. If a table is used, do not use a pillow because this elevates the person's head and may contribute to vertigo (dizziness). Try to make the donor comfortable and avoid contributing to any mental anxiety he may have. A calm professional manner is in order. Casual conversation is acceptable, but do not tell the patient he has "bad veins" or otherwise suggest that he may have difficulty. The phlebotomist who repeatedly asks such questions as "Are you sure you feel all right?" is psychologically encouraging the donor to react.

7-25. At this point, the donor is comfortable, and all of your equipment is in place. The room should be about 72° F. and well ventilated. It is better to keep the temperature cool rather than overly warm. Female donors should be covered with a sheet over their legs. Keep the donor area clean and free from blood stains. Though spattered blood does not bother an experienced technician, it can be repulsive to the average person. Finally, before you begin to draw the blood, be certain that a physician is in the building and can be summoned quickly. At least one other person must be within calling distance so that you can summon aid if the donor develops a reaction.

7-26. The suggested cleansing of the phlebotomy site includes the use of surgical soap, 70 percent isopropyl alcohol, and tincture of Merthiolate, in that order. Swab the skin in a rotary fashion, holding the 2 x 2 gauze pad or cotton with a forceps and moving from the intended venipuncture site to the periphery. Scrub with firm pressure and repeat with the first 2 x 2 pad until it no longer appears "dirty" before using the alcohol and Merthiolate. It is very important to clean the site thoroughly to prevent contamination of the unit or infection of the donor. As we mentioned, handle gauze and cotton pads with a forceps. Some technicians have the very bad habit of using their fingers to apply the pads, and what is worse, squeezing excess solution back into the container of antiseptic. This act rinses bacteria from their hands into the stock container. (Remember, most so-called antiseptics do not eradicate bacteria immediately upon contact. *Staphylococcus* organisms can survive for long periods in 70 percent alcohol.) After cleansing the skin, do not touch the venipuncture site unless you swab it again.

7-27. You are probably familiar with the phlebotomy technique. There are three basic considerations in collecting blood. First, how to draw the blood; second, what to do if a donor reaction occurs during the drawing process; and third, how to stop the blood flow. To refresh your memory, we will list some of the steps in starting the flow of blood.

a. Make a loose overhand knot in the integral donor tubing of the blood pack, 6 or 8 inches from the needle.

b. Suspend the blood pack on the previously prepared support and apply the tourniquet. If a blood pressure cuff is used, inflate the pressure to 60 mm. of mercury and maintain this pressure throughout the phlebotomy.

c. Grasp the needle hub firmly, twist the cover to break the seal, then pull the cover off.

d. Perform the venipuncture. Insert the needle well into the vein. (The needle can be secured in position with adhesive tape.)

e. Release the bead at the base of the donor tube to permit blood flow into the collecting bag. Squeeze the tubing above the bead between thumb and forefinger to force the bead completely free into the blood pack.

f. Mix the blood and anticoagulant. Mixing should begin as soon as the blood flow has been established. Gently elevate the bottom of the blood pack several times at intervals during collection to insure thorough mixing. Avoid tension of the tubing because this might disturb the needle in the vein.

7-28. Occasionally a donor develops a convulsive-like reaction, and you need to be prepared. If this reaction does occur during the phlebotomy, remove the needle and protect the donor from biting his tongue by using a tongue depressor. Prevent the donor from falling off the table or suffering other injury. Usually, elevation of his feet, a cool towel on his forehead, and other simple measures will resolve the problem, but the physician in attendance should be consulted.

7-29. If there is *no* abnormal donor reaction, then stop the blood flow in the following sequential steps:

a. Release the pressure in the cuff. Retain the pressure cuff in position for possible use when taking the pilot tube samples.

b. Apply a hemostat about 4 inches below the needle just above the prepositioned knot. Tie the knot tightly and sever the tubing between the knot and the hemostat. Avoid disturbing the needle when tying the knot. Blood in the integral pilot tubing can be "stripped" into the bag if plasma is desired. Do not allow blood to reflux out of the bag while the end of the tube is open; to do so is a break in sterile technique.

c. You can collect additional pilot tubes from the cut end of the donor tube (still inserted into the donor's vein) by releasing the hemostat. Pressure may be reapplied in the pressure cuff, if necessary, to obtain the desired amount of blood for samples.

d. Be sure the tourniquet pressure is released.

e. Exert gentle pressure with a sterile sponge immediately above the site of venipuncture and withdraw the needle. Elevate the donor's arm vertically without flexing the elbow and have him apply firm pressure on the sponge to prevent blood leakage from the vein and consequent hematoma formation.

f. Apply a sterile dressing over the site of puncture.

g. Allow the donor to rest 10 to 15 minutes.

7-30. Label each unit of blood promptly with the following: unit accession number, expiration date, blood group, results of a screen for atypical antibodies, and Rh type. To be considered a true negative, the blood must be negative for CDE and D^u. Refrigerate blood immediately after it is drawn.

8. Blood Storage and Shipment and the Air Force Blood Program

8-1. All biologicals are subject to deterioration if they are not properly stored. Blood is not only a biological, it is a suspension of metabolically active cells and delicate platelets. The blood has been removed from the circulatory system with its associated means of supplying the cells with metabolites and removing waste products. Refrigeration of whole blood is necessary to reduce cellular activity and proliferation of bacteria. Many of the storage requirements and additional problems must be considered when blood is shipped. We will mention some of the most significant features of storage and shipment in this section and describe the military blood program, which has become a matter of wide interest because of the conflict in Southeast Asia. Material contained in our discussion of the military blood program is current as of this writing. It varies somewhat from information contained in earlier publications and undoubtedly will be updated, as this highly flexible and rapidly changing program is adjusted to meet new requirements.

8-2. **Routine Storage of Blood.** Blood must be stored at a temperature of 1° C. to 6° C., and variation within this range must not be more than 2° C. Blood should be stored in a refrigerator that is coupled to a temperature recording device and provided with an alarm system, which is activated if the proper temperature is not maintained. The thermostat controlling this alarm must be a double point system, registering temperatures both above and below the range. The alarm system should sound in an area where some responsible person is on duty 24 hours a day. If this person is not part of the blood bank staff, he must be briefed on what to do if the alarm sounds. Alarm systems must be battery operated. Batteries equipped with trickle chargers are available for this purpose. In addition to an alarm system, a recording thermometer providing a permanent record of temperature fluctuations is desirable. Blood must not be stored in refrigerators on wards or other places where the refrigerators do not meet all of the criteria for a blood bank refrigerator. Blood-storage refrigerators are used only for blood, blood products, typing serums, pilot tubes, or reagents directly related to blood banking. Never use a blood bank refrigerator for storing bacteriology media, food, or any material not related to blood banking. Only specially designed commercial blood bank refrigerators should be used, except where field conditions prevail. Outdated blood must be promptly removed from the blood-bank refrigerator so that it will not be inadvertently administered to someone.

8-3. **Frozen Blood.**[1] The development of techniques for successfully freezing and thawing red blood cells was the greatest advancement in blood banking technique since the institution of ACD (acid-citrate-dextrose) preservative.[2] It changed the concept from one of "delayed deterioration" to one of true preservation for a prolonged period. The use of ACD and other nutrient-holding solutions allows a shelf life (70 percent survival) of 3 weeks for unfrozen blood. After that, if the blood has not been used, it is discarded. It is estimated that between 500,000 and 1,000,000 units of blood become outdated each year in the United States, and salvaging these units would certainly solve many problems in blood procurement.

8-4. Multiple techniques of freeze preservation are being developed, and constant improvements are being incorporated into the techniques. Since 1949, when Polge, Smith, and Parkes[3] developed a successful viable preservation of fowl spermatozoa frozen in glycerol and stored at −79° C., the application of freezing techniques to the preservation of blood has generally centered around the investigation of freezing rates and solutions to find those giving the most satisfactory results.

8-5. The freezing rate segregates the methods into two broad categories. *Rapid freeze techniques* lower the temperature several hundred degrees per minute by the use of liquid nitrogen at −197° C., with freezing in 60 to 75 seconds.

8-6. *Slow freeze techniques* use dry ice in ether (−75° C.) or refrigeration by deep freeze (−80 to −85° C.), with a freezing time of 2 to 3 hours.

8-7. The technical considerations of the changes produced by freezing and thawing are extensive and will not be discussed in detail in this course. However, points to be considered include: the effect of ice crystal formation on the cell membranes, cytoplasm and nuclear material; osmotic effect due to concentration as the "cell water" freezes; binding of water by additives that either act only on the surface or penetrate into the cell; and the technique and effect of the removal of additives.

8-8. The method that has recieved the greatest clinical usage was developed by Dr. Charles E. Huggins at Massachusetts General Hospital in conjunction with the U.S. Navy. His method was subsequently evaluated at USAF Hospital, Clark Air Force Base, and at medical facilities in Southeast Asia. This method produces a unit of washed red cells with a survival rate of 87 to 95 percent. Cells are washed free of nearly all undesirable chemicals, leukocytes, platelets, isoagglutinins and metabolic products.

8-9. The method of preparation includes the following manipulations. Blood is collected in a routine way in standard double plastic bags with ACD preservative (NIH formula A). Typing and serologic examinations are done. Within 5 days the blood is centrifuged and the plasma expressed into the second part of the double plastic pack. The cells are transferred to a plastic freezing unit and glycerolized by the addition of an equal volume of 8.6 M glycerol in 8 percent glucose—1 percent fructose—0.3 percent EDTA. The blood and glycerol are mixed and pilot tubes collected.

8-10. The blood is frozen at −85° C. and held until needed. It has a proven shelf life of 2 years and may possibly be stored as long as 10 years. The blood is thawed in a 40° C. water bath, deglycerolized and washed in a Huggins cytoglomerator, Model WS. The wash phase of the preparation includes a first dilution with 50 percent glucose, which osmotically removes a large portion of the glycerol. This is followed by three subsequent washes with 6000 ml. of 8 percent glucose and 1 percent fructose. The nonelectrolytic solution causes the red cells to agglomerate, permitting cells and solution to separate without the aid of centrifugation. The final addition of an electrolytic solution, normal saline, causes the erythrocytes to become individually resuspended. The preparation may be administered either as washed cells in saline or as packed cells. Processing time is only 20 minutes per pack, and 5 units can be processed simultaneously on the Huggins cytoglomerator.

8-11. The clinical application of the frozen blood at Clark Air Force Base has shown it to be highly effective and a superior source of blood for special cases. The total usage has not been large—215 units from December 1966 to June 1967. This represents an overall average usage of 4.4 percent per month with use in some months rising to 9.0 percent. The frozen blood is a good backup for regular ACD blood requirements and is particularly helpful when packed red cells are needed. When used in large quantities, frozen blood must be supplemented with fresh blood to supply essential coagulation factors. Platelets have been found to be decreased in cases receiving large quantities of frozen blood, but bleeding has not been a great problem. The decrease in platelets is expected in view of the numerous dilution procedures used in preparing frozen blood.

8-12. An additional advantage for patients recieving a large number of transfusions is the reduction of acidosis and anticoagulant effect of ACD in conventionally stored blood. Frozen blood has been found very helpful in transfusing patients

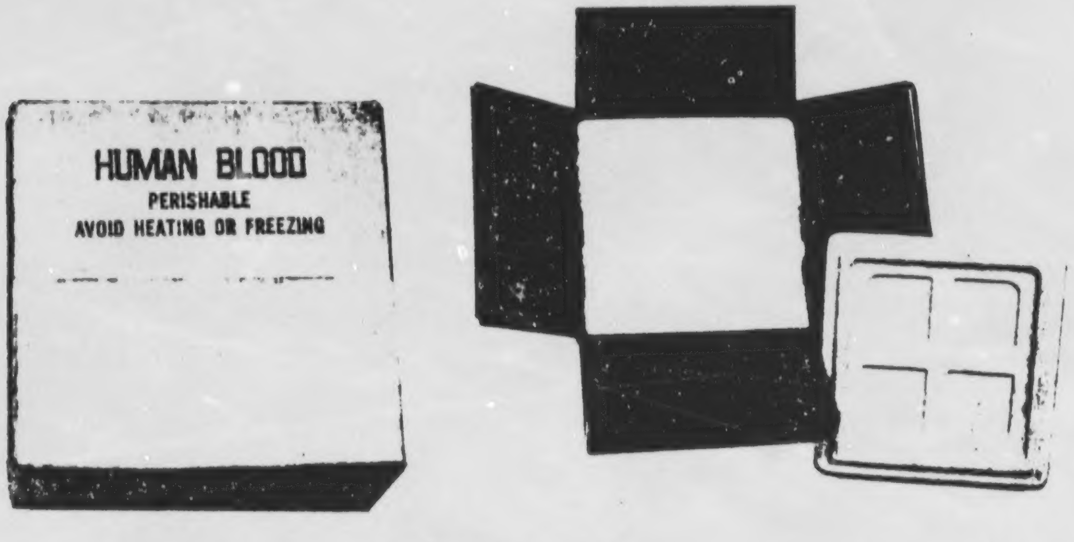

Figure 9. Blood shipping container.

with a rare blood type for which no donor is locally available.

8-13. **Shipment of Blood.** Blood must be kept between 1° C. and 10° C. during shipment. Double-wall cardboard boxes or cardboard boxes with specially designed styrofoam inserts are used. Examples are shown in figure 9. The boxes are clearly labeled "Human Blood." Never ship blood in a container labeled otherwise. The best way to ship blood any distance is by air. Ordinary ice is used, not dry ice. The ice is placed in one or two plastic bags and tied securely. Ideally, the ice pack should not touch the blood bags directly, because this increases the chances of damage and hemolysis. AFM 160-50, AFR 160-26, and AFM 168-3 contain detailed instructions for shipping blood.

8-14. A shipping record is a necessary part of transporting blood. Use DD Form 573 (Shipping Inventory of Blood Collections) when shipping blood through DOD blood program channels. DD Form 573 or a similar form is usually required when blood is shipped to private blood banks or other facilities. Keep the following points in mind:

a. If you ship and receive blood, you must account for each unit.

b. Pay particular attention to expiration dates. If a unit of crossmatched blood is to be returned to a lending blood bank, it may be necessary to either release the unit or crossmatch another unit. Blood that is not shipped on time is usually "charged off" to the facility concerned. Blood is too expensive and difficult to obtain to permit waste! Even more important, a medical problem arises if expired blood is held for a patient. Obviously, you cannot give expired blood.

c. The DD Form 573 is a valuable document. It provides continuity in identifying the units with other records at the collecting and receiving centers. The form may also help trace the blood or establish liability if a shipment is lost in transit. A copy of DD Form 573 is printed in foldout 2.

8-15. Blood should not be released to the carrier prior to 1 hour before its departure. If the scheduled train, bus, or plane departure is delayed after the blood has been released to the carrier, the carrier should contact the transportation officer. To insure shipment, it has often proved necessary for a representative from the blood bank to remain with the blood until it has departed. It is also wise to notify the recipient by AUTOVON or other rapid means when a shipment is scheduled to arrive. Blood containers should never be exposed to extreme temperatures. Blood must not be placed in the lower compartment of planes or in any place where the ambient temperature falls below 32° F. If there is delay enroute, the carrier may be instructed to break the seal in order to re-ice. If this is done, a signed statement from the carrier agent who breaks the seal, indicating the time and date re-iced, must be placed in the container for the receiver's information. Wet ice *only* is used for re-icing. (Dry ice lowers the temperature too much.) The receiver must not accept a container of blood with a broken seal unless the explanation is signed by the carrier. In such a case, the receiver should notify the officer in charge of the donor center immediately.

8-16. Blood must be transported as expeditiously as possible. Every attempt must be made to have blood at its destination the same day it is shipped. Processing laboratories (for overseas blood shipments) receive blood 24 hours a day; therefore, the local carrier should not hold containers of blood overnight for delivery the next day. We will discuss these processing laboratories in the following paragraphs.

MODIFICATIONS

Section 8-17 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.

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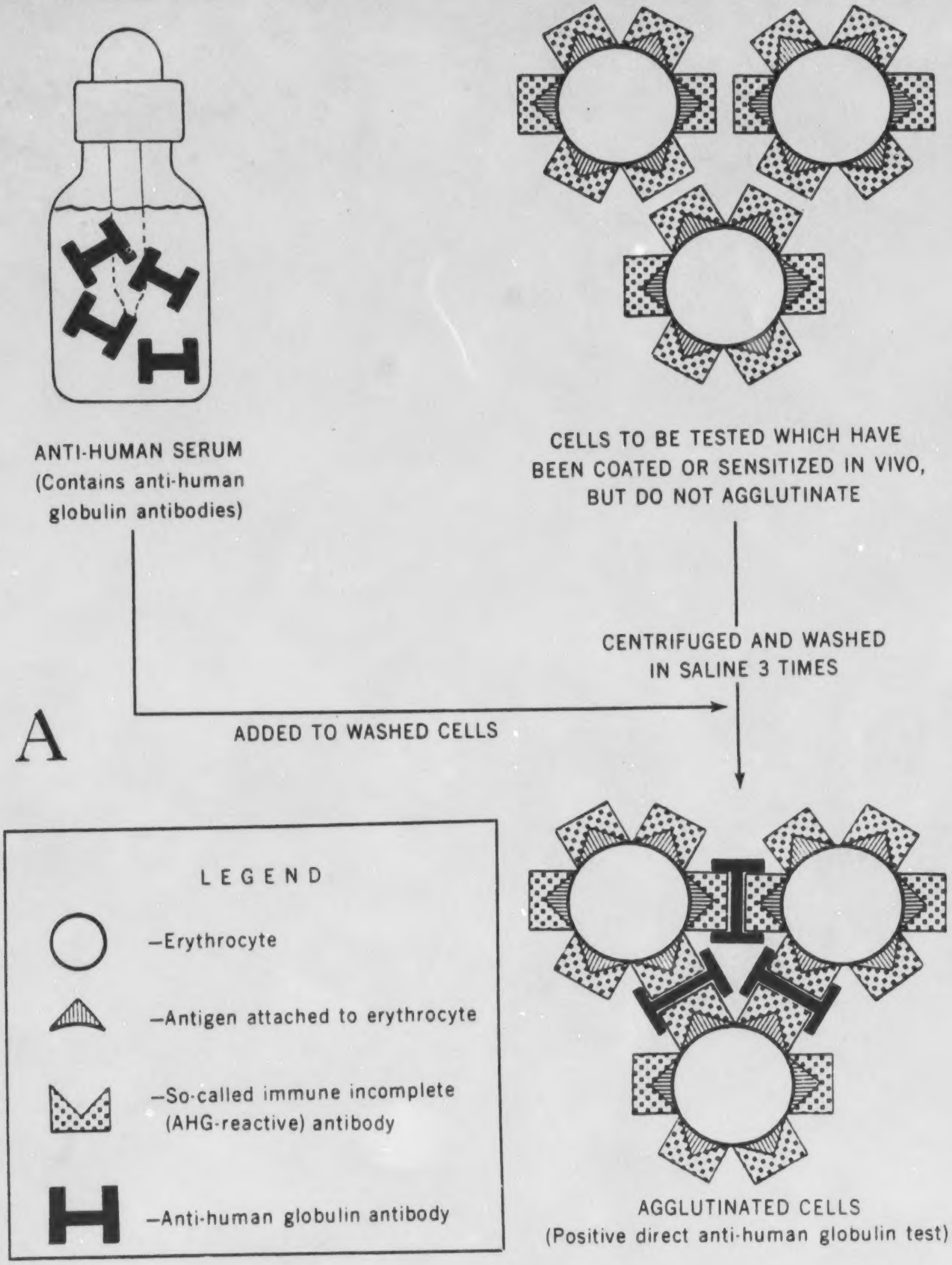
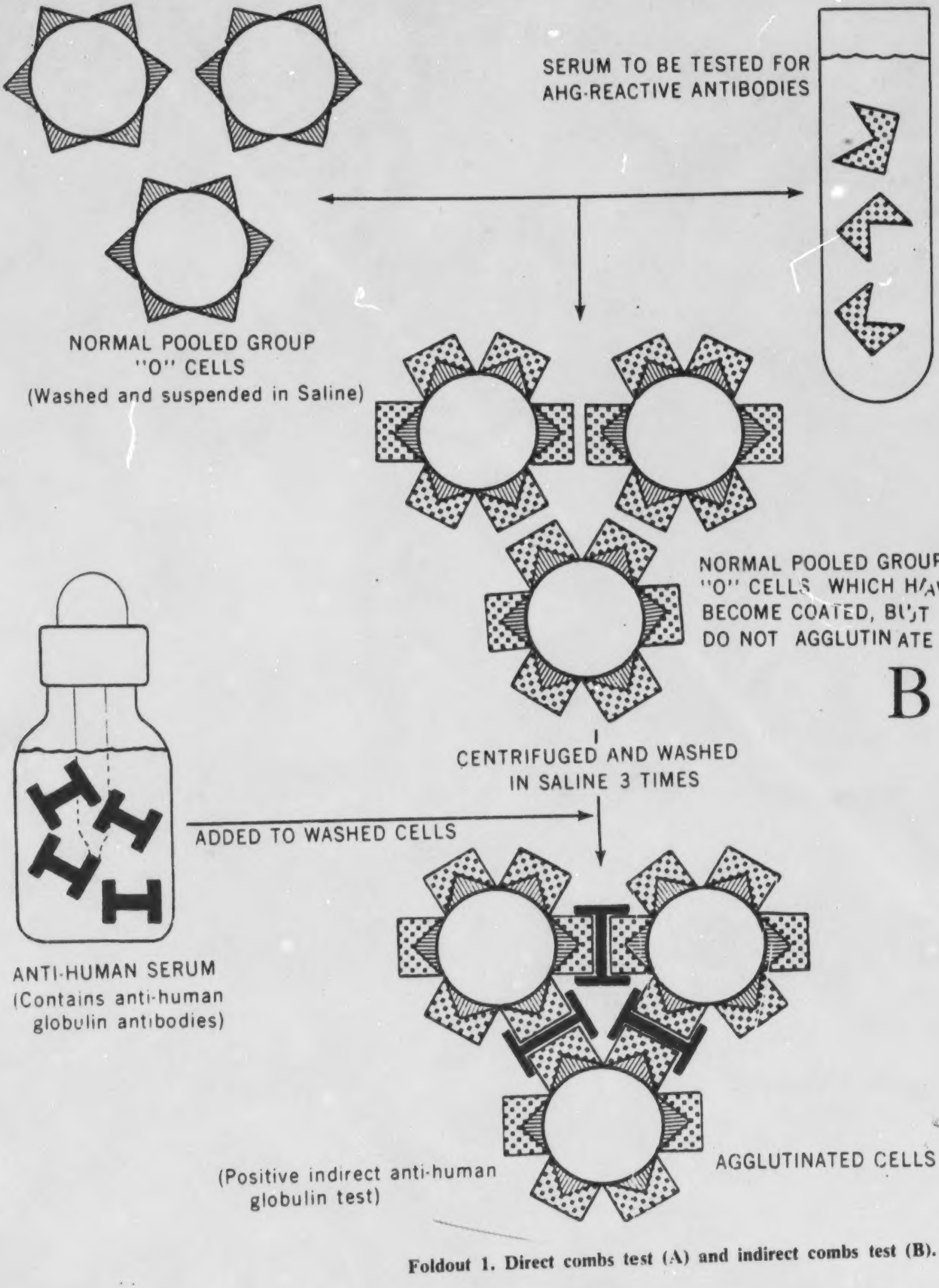

ANTI-HUMAN SERUM
(Contains anti-human globulin antibodies)
CELLS TO BE TESTED WHICH HAVE BEEN COATED OR SENSITIZED IN VIVO, BUT DO NOT AGGLUTINATE
CENTRIFUGED AND WASHED IN SALINE 3 TIMES
ADDED TO WASHED CELLS
A
LEGEND
–Erythrocyte
–Antigen attached to erythrocyte
–So-called immune incomplete (AHG-reactive) antibody
–Anti-human globulin antibody
AGGLUTINATED CELLS
(Positive direct anti-human globulin test)

Foldout 1. Direct combs test (A)


SERUM TO BE TESTED FOR
AHG-REACTIVE ANTIBODIES
NORMAL POOLED GROUP
"O" CELLS
(Washed and suspended in Saline)
NORMAL POOLED GROUP
"O" CELLS WHICH HAVE
BECOME COATED, BUT
DO NOT AGGLUTINATE
B
CENTRIFUGED AND WASHED
IN SALINE 3 TIMES
ADDED TO WASHED CELLS
ANTI-HUMAN SERUM
(Contains anti-human
globulin antibodies)
AGGLUTINATED CELLS
(Positive indirect anti-human
globulin test)

Foldout 1. Direct combs test (A) and indirect combs test (B).

MODIFICATIONS

Foldout 2 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.

10-4

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WORKBOOK
LABORATORY PROCEDURES IN BLOOD BANKING AND IMMUNOHEMATOLOGY

SELF-TEACHING AIDS
CRE
VRE
SRG

This workbook places the materials you need *where* you need them while you are studying. In it, you will find the Study Reference Guide, the Chapter Review Exercises and their answers, and the Volume Review Exercise. You can easily compare textual references with chapter exercise items without flipping pages back and forth in your text. You will not misplace any one of these essential study materials. You will have a single reference pamphlet in the proper sequence for learning.

These devices in your workbook are autoinstructional aids. They take the place of the teacher who would be directing your progress if you were in a classroom. The workbook puts these self-teachers into one booklet. If you will follow the study plan given in "Your Key to Career Development," which is in your course packet, you will be leading yourself by easily learned steps to mastery of your text.

If you have any questions which you cannot answer by referring to "Your Key to Career Development" or your course material, use ECI Form 17, "Student Request for Assistance," identify yourself and your inquiry fully and send it to ECI.

Keep the rest of this workbook in your files. Do not return any other part of it to ECI.

EXTENSION COURSE INSTITUTE

Air University

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ECI Form No. 17

STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume. Use the Guide for review before you take the closed-book Course Examination.

2. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the items missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Numbers 200 through 206

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CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 1

Objectives: To display a knowledge of the basic principles of the laws of genetics as they apply to the inheritance of various blood group systems; to demonstrate an understanding of the immune response to foreign blood group antigens and methods of detection and prevention.

1. (a) Give a simple definition of genetics.

   (b) What part do genes play in our inheriting certain characteristics?
   (1-1)

2. Distinguish between *phenotype* and *genotype*. (1-2)

3. (a) How do the chromosomes in females differ from those in males?

   (b) What determines the sex of an offspring?
   (1-4)

4. (a) What is a sex-linked gene?

   (b) How many genes would a male have for a given sex-linked trait?

   (c) How many could a female have?
   (1-4, 5)

5. List all the possible genotypes for the ABO blood groups. (1-6, 7)

6. (a) What are alleles?

(b) How many genes for ABO blood group are there on a single chromosome? (1-7)

7. Assuming that a mother's Rh gene type is CdE/cdE and the father's genotype is cDE/cde, what are the possible genotypes of an offspring? (1-8)

8. When we genotype a person for D and report his genotype as heterozygous Dd, how do we test for the d gene? (1-9)

9. Match the words in the column on the left below with the selection that it best fits on the right. (1-12-17)

A.	Antigen	(a)	Gamma globulin
B.	Combining site	(b)	Hemolytic disease of the newborn
C.	Immune response	(c)	Determinant
D.	Isoimmunization	(d)	ABO antibody
E.	Natural antibody	(e)	Rh$_o$ (D)
F.	Isoantigen	(f)	Antigenic stimulation
G.	Antibody	(g)	High molecular weight protein

10. What kind of substances are IgA, IgG, and IgM? (1-17)

11. List five variants of group A antigens. (1-18)

12. What two types of antigen-antibody reactions are usually seen in the blood bank? (1-19)

13. What do cold agglutinins most frequently cause? (1-21)

14. Why should you carefully control centrifuge speed when you are spinning cells? (1-23)

15. How does too much antigen or antibody affect antigen-antibody reactions? (1-24)

16. List several complement-fixing antibodies that may be encountered in the blood bank. (1-25)

17. Why does the use of enzymes on cell preparations sometime give misleading readings? (1-25)

18. How do complement-fixing antibodies affect cell survival within the body? (1-26)

19. (a) What is the most frequent cause of hemolytic disease of the newborn?

(b) Name the immunological tests used to detect and monitor this condition.

(c) What is the name of the preparation now used to prevent this disease?
(2-1-3)

20. What are the two primary benefits of an exchange transfusion? (2-7)

21. Why must blood used for an exchange transfusion on an infant be compatible with the mother's serum? (2-8)

22. If you crossmatch a vial of Rh_o human immune globulin against a patient and the test shows agglutination, what should you do next? (2-12)

23. At what temperature should Rh_o human immune globulin be stored? (2-19)

CHAPTER 2

Objectives: To demonstrate a knowledge of the systems of grouping and identifying blood and the methods used to differentiate between them, and to be able to compare and show the relationships between the various systems.

1. Who discovered the ABO system? (3-1)

2. What do we mean when we say ABO antibodies are "naturally occurring"? (3-3)

3. What stimulates the production of ABO antibodies? (3-3)

4. Which is the most common blood group? (3-4; Table 3)

5. What is the precursor substance that, under genetic stimulation, produces A or B antigen? (3-5)

6. Why do group O cells contain more H substance than A or B cells? (3-5)

7. What kind of substance is H substance? (3-5)

8. What is unusual about the rare O_h (Bombay) blood? (3-5)

9. Give two methods of detecting subgroups or weak A and B antigens. (3-6)

10. List sources of A and B antigens other than red blood cells. (3-8)

11. What gene controls the secretion of A and B substances into the body's fluids? (3-8)

12. Why should blood grouping tests on transfusion blood always be performed by the tube method? (3-9)

13. List some sources of error for ABO grouping. (3-9)

14. What other names mean the same as serum grouping? (3-10)

15. Why must we perform serum grouping tests on all donors and recipients? (3-10)

16. In the chart below show the serum grouping reaction with each of the four different kinds of cells. (3-11, 12; Table 4)

	Test Cells			
	A_1	B	O	A_2
Group A				
Group B				
Group AB				
Group O				
Group A_2				
Group A_2B				

17. Which blood group is most often mistyped? What procedure will usually eliminate this error? (3-11)

18. Why should test cells for serum grouping procedures be prepared fresh each day? (3-12)

19. Why are group O cells used in the serum grouping procedure? (3-12)

20. What are the two main sources of Lewis antigens? (3-14)

21. Of the two Lewis phenotypes, which is the more common? (3-15)

22. What type of immunoglobulin is Le^a? Le^b? (3-16)

23. Name two systems used to classify Rh antigens and antibodies. (4-2)

24. An antiserum is not available for which of the Rh antigens? (4-3)

25. On typing tests, a "true Rh negative" must be negative for what factors? (4-3)

26. What is the result of the D^u test if the blood is Coombs positive? (4-4)

27. How many different kinds of antigens can be present on a red blood cell? (4-6)

28. Match the antigen on the left below with its symbol on the right. (4-7-10)

A. Kell	(a)	JK^{a+b}
B. Duffy	(b)	Lu^{a+b}
C. Kidd	(c)	Kk
D. Lutheran	(d)	Fy^{a+b}

29. Distinguish between "private" and "public" antigens. (4-13)

CHAPTER 3

Objectives: To be able to isolate and identify antibodies, using the Coombs and other antibody detection procedures; and to demonstrate a knowledge of the operation of a hospital blood bank.

1. What are the two most significant factors in antigen antibody reactions? (5-2)

2. What is anti-human globulin (AHG) commonly called? (5-3)

3. In the direct Coombs test, where are the antibodies we search for? (5-3)

4. Name the three animals most often used to produce Coombs serum. (5-4)

5. What antigen is used to stimulate animals in the production of Coombs serum? (5-4)

6. Why is it necessary to blend serum from several different animals in producing Coombs serum? (5-4)

7. Will using your finger or thumb instead of a stopper cause the Coombs test to be falsely positive or negative? (5-5)

8. How is the reactivity of Coombs serum affected if it is left at room temperature? (5-5)

9. What drug is cited as producing false postive Coombs test during therapy? (5-7)

10. List three steps in antibody studies. (5-8)

11. How does antibody detection differ from antibody identification? (5-8,9)

12. List three ways in which "nonnaturally occuring" antibodies are stimulated in humans. (5-9)

13. Why are commercially prepared reference cells more desirable than "homemade" ones? (5- 10)

14. What type of antibody is detected if incubation at 4° C. is used? (5-12)

15. If a control composed of the patient's own cells and serum is run along with the Coombs test, what type of reaction can be detected? (5-12)

16. What is a cell panel? (5-13)

17. If your tests produce different reactions in different media in the antibody identification test, what does this indicate? (5-13)

18. Define dosage. (5-13)

19. If immune anti-A is suspected, how can you detect it? (5-14)

20. When should you use enzymes in antibody screening procedures? (5-15)

21. What is the most critical property in the use of enzymes? (5-15)

22. What are the two procedures that are used to remove antibodies from serum or from red cells? (5-16, 17)

23. How does calculating titers of antibody solutions in the blood bank differ from calculating titers for general serological procedures? (5-18)

24. Which tube or dilution is reported as the titer? (5-18)

25. Though platelet transfusion is a major advance, what is the danger in its overuse? (5-19)

26. List the advantages of keeping good records in the blood bank. (6-3)

27. Name three vital record documents used in the blood bank. (6-5-8)

28. How would you report a crossmatch test that shows hemolysis? (6-13)

29. Name the media used in crossmatch procedures. (6-13)

30. What is the primary purpose of a crossmatch procedure? (6-14)

31. What errors do crossmatch procedures fail to detect? (6-15)

32. When can you release uncrossmatched blood for transfusion? (6-19-21)

33. What substances is the blood bank allowed to add to blood crossmatched for transfusion? (6-23)

34. List some reactions that a patient might experience as a result of receiving incompatible blood. (6-29)

35. *In vivo* agglutination is likely to result in what kind of transfusion reaction? (6-31)

36. What are the two most dangerous forms of transfusion reaction? (6-31-33)

37. What disease is most often transmitted by transfusion? (6-34)

38. Name the particle that is transmitted during transfusion therapy and is thought to cause serum hepatitis. What sort of particle is it? (6-36)

39. List six methods for detecting the particle associated with serum hepatitis. (6-37)

CHAPTER 4

Objective: To demonstrate an understanding of the criteria for blood donor selection and blood collection, and the processing, shipping and storage of blood, with emphasis on the Military Blood Program.

1. Why should the technician establish a good relationship with the blood donor? (7-1)

2. As a blood collector, you have the responsibility of checking to be sure a blood donor is eligible to give blood. Where can you find the information you need? (7-2,3)

3. List some of the physical examination information found on DD Form 572, Blood Donor Record Card. (7-2-4)

4. Besides providing information about the donor, how else can the Blood Donor Record Card be of value? (7-4)

5. From the point of view of patient comfort, why is it advisable to have blood donors eat a nonfatty meal prior to giving blood? (7-7)

6. Name three infectious diseases that permanently exclude donors from giving blood. (7-9, 10)

7. If you are screening blood donors and one has a history of frequent upper respiratory infections, what should you do? (7-13)

8. Do you think a donor who is still experiencing a reaction 3 weeks after inoculation is acceptable? (7-14)

9. Suppose your blood bank needed blood badly and a male donor showed up and had a 13.2 gm. hemoglobin. Would you use him? (7-15)

10. What is the first thing to do when a donor reports to your table to give blood? (7-24)

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11. Why is alcohol alone not enough for preparing the phlebotomy site? (7-26)

12. Why should blood be refrigerated as soon as possible after collection? (8-1)

13. What is the maximum variation in temperature allowed for stored blood? (8-2)

14. Why must alarm systems be battery operated or connected to an emergency power outlet? (8-2)

15. List three good reasons why blood should *never* be stored in a ward refrigerator. (8-2)

16. Name a new technique that may revolutionize blood storage and utilization. (8-3-5)

17. What is the difference between the rapid freeze and the slow freeze technique for preserving blood? (8-5, 6)

18. What are some of the advantages to the patient when frozen blood is used for transfusions? (8-12)

19. How are boxes used to ship blood labeled? (8-13)

20. When is the use of DD Form 573, Shipping Inventory of Blood Collections, mandatory? (8-14)

21. Can dry ice be used to keep blood cool during regular shipment? (8-15)

22. Who administers the military blood program? (8-19)

23. In the Military Blood Program, where is the collected blood sent for processing? (8-26)

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ANSWERS FOR CHAPTER REVIEW EXERCISES

CHAPTER 1

1. (a) Genetics is the study of heredity and inheritance.
   (b) Genes determine all the physical, chemical and structural properties of the body, including the various antigens in our red blood cells.

2. Phenotype is the physical expression of a trait. Genetically, several members of a group may possess a similar trait. This trait, however, may be due to the inheritance of differing combinations of genes by each individual exhibiting the trait. A good example of phenotype is those individuals whose blood we type as group A. This type of blood is found in individuals who inherit either the AA or AO gene combination. Either combination causes the individual to type as group A. Consequently, we say their phenotype is "A." On the other hand, genotype refers to the actual gene combination that is inherited. In each case of a group A individual, the genotype may be AO or AA. We cannot test for this property. In order to determine an individual's genotype, we must phenotype several members of a family, (i.e., mother, father, grandparents, etc.) and calculate the genotype according to Mendelian principles of inheritance.

3. (a) A females' body cells contain two X chromosomes. When ova are produced by reduction division (meiosis), each cell will carry one X chromosome. Males have an X and a smaller Y chromosome. When spermatozoa are produced, they may carry either an X or a Y chromosome.
   (b) If a spermatozoan carries an X chromosome and fertilizes an ovum, the offspring will be female. On the other hand, if a spermatozoan carries a Y chromosome and fertilizes an ovum, the offspring will be male.

4. (a) A sex-linked gene is a gene that occurs on X chromosomes only.
   (b) A male can have only one sex-linked gene for a particular trait.
   (c) Females, because they have two X chromosomes, may carry two sex-linked genes.

5. Genotypes for the ABO blood groups:
   A – AA and AO
   B – BB and BO
   AB – AB
   O – OO

6. (a) Alleles are pairs of genes. One of this pair is inherited on a chromosome in the ovum; the other is inherited on an identical chromosome in the spermatozoan. A male sometimes manifests a trait for which he has only one gene. This occurs because that gene is found on the X chromosome; there is no allele or mate to it on the Y chromosome.
   (b) There is only one A, B, or O gene on a single chromosome.

7. This offspring may have the following Rh genotypes:

   (a) CdE/cde
   (b) CdE/cDE
   (c) cdE/cde
   (d) cdE/cDE

8. We don't test for the d gene. In order to determine its presence or absence, we must do family studies to determine the presence or absence of D. In the absence of D, we assume that d is present.

9. A. (g), B (c), C (f), D (b), E (d), F (e), G (a).

10. Immunoglobulins.

11. Five variants of group A antigen are A_1, A_2, A_3, A_m, A_x.

12. The blood bank technician usually observes antigen-antibody reactions as agglutination and hemolysis.

13. Autoagglutination.

14. The centrifuge speed must be carefully controlled because spinning too fast packs the cells too tightly, which may produce false agglutination. Spinning too slowly does not force the cells closely enough together and may result in a false-negative reading.

15. Too much antigen or antibody can result in weak or negative tests.

16. Some complement-fixing antibodies are anti-A, anti-B, anti-le^a , anti-le^b, anti-Jk^b, anti-K and anti-Fy^a.

17. Enzymes sometimes alter antigens on the cell surface. When tested, these cells may give misleading results.

18. Cells coated with complement-fixing antibodies are removed from the circulation and destroyed much faster than noncoated cells.

19. (a) Rh isoimmunization.
    (b) Direct and indirect Coombs test.
    (c) Rh_o (D) human immune globulin (Rh_oGam: Ortho Pharm. Co.)

20. (a) Replaces destroyed erythrocytes.
    (b) Removes antibodies from the circulation.

21. Because the blood must be compatible with the antibodies in the mother's serum. These antibodies cross the placenta and coat the baby's cells. The coated cells are rapidly destroyed within the baby's circulatory system.

22. Get a vial with a different lot number and repeat the crossmatch.

23. At normal blood bank refrigerator temperature (4° to 6° C.)

CHAPTER 2

1. Karl Landsteiner.

2. "Naturally occurring" means that these antibodies occur without the stimulation of transfusion, injection, or pregnancy.

3. ABO antibodies are stimulated by substances in food, water, and air that have antigenic properties similar to those of A and B antigens.

4. Group O.

5. H substance.

6. The O gene does not stimulate or change H substance; therefore, the amount of H substance in the cell does not diminish. In the case of A and B cells, when A or B antigen is produced, the H substance is mostly used up.

7. A mucopolysaccharide.

8. The cells of these group O individuals do not contain any H substance; hence, they can develop anti-H as well as anti-A and anti-B.

9. (a) Elution.
   (b) Lectins (plant agglutinins).

10. (a) Leukocytes.
    (b) Platelets.
    (c) Saliva.
    (d) Seminal fluid.

11. The Se gene.

12. Because tube testing is more reliable.

13. (a) Contaminated and impotent antisera.
    (b) Cold agglutinins.
    (c) Other saline reactive agglutinins.
    (d) Polyagglutinable erythrocytes.

14. (a) Proof grouping.
    (b) Reverse grouping.
    (c) Back grouping.

15. Serum grouping is a quality control procedure and, when performed, insures that we have correctly grouped the blood being tested.

16.

	Test Cells			
	A_1	B	O	A_2
Group A	-	+	-	-
Group B	+	-	-	+
Group AB	-	-	-	-
Group O	+	+	-	+
Group A_2	(+)	+	-	-
Group A_2B	(+)	-	-	-

17. (a) Subgroups of A.
    (b) Serum grouping.

18. Red blood cells suspended in saline deteriorate rapidly and must be prepared fresh each day.

19. To detect autoagglutination.

20. Saliva and serum.

21. Le^b.

22. (a) IgG.
    (b) IgM.

23. (a) Fisher-Race.
    (b) Wiener.

24. There is no anti-d (Hr_o) antiserum available.

25. A true Rh negative must be negative for C, D, E, and D^u.

26. The result is always D^u positive and therefore invalid.

27. Thousands.

28. (a) Kell - Kk.
    (b) Duffy - Fy^a + ^{b}.
    (c) Kidd - Jk^a +^{b}.
    (d) Lutheran - Lu^a +^{b}.

29. "Private" antigens are rare antigens that cause sensitization in very rare instances. Consequently, antibodies against these antigens are not encountered vey often. "Public" antigens are also rare antigens, but, once they stimulate antibody response, these antibodies will agglutinate most other red cells. It is very difficult to find blood compatible for a person sensitized with a public antigen.

CHAPTER 3

1. (a) Repelling electrical charges.
   (b) Dimensions of the antibody molecule.

2. Coombs serum.

3. In the direct Coombs test we seek to detect antibodies that have coated the patient's cells.

4. Rabbits, goats, and sheep.

5. Human gamma globulin.

6. Each animal's response to an injection of human gamma globulin is different. In order to get maximum reactivity and for standardization of the product, it must be blended.

7. Falsely negative.

8. Coombs serum deteriorates rapidly at room temperature.

9. α-methyldopa.

10. Detection, titering, and identification.

11. Antibody detection is a qualitative screening procedure that shows the presence of antibodies in a serum specimen. Antibody identification involves testing the serum against known antigens to identify the antibody accurately.

12. (a) Previous transfusions.
    (b) Parenteral injections of blood.
    (c) Isominnunization in pregnancy.

13. Commercially prepared reference cells usually contain a greater variety of antigens than locally prepared solutions. This is especially true for the semirare and unusual antigens. It is also inadvisable legally to make your own reagents.

14. Cold-reacting antibodies.

15. Autoagglutination.

16. A cell panel is a series of vials of different known antigens used in testing.

17. The presence of more than one type of antibody.

18. Dosage is a condition in which cells from a person possessing a homozygous gene combination tend to react more strongly with specific antibodies than the cells of those persons with a heterozygous gene combination.

19. You can detect immune anti-A by adding blood group specific substance A to the serum specimen. This neutralizes the naturally occurring anti-A. The specimen is then tested in the usual way for detecting antibodies.

20. Enzymes should be used only in those cases where there is a good probability of the presence of an enzyme reactive antibody.

21. pH.

22. Adsorption and elution.

23. The volume of antigen suspension (cells) is not used in calculating titers in the blood bank.

24. The last tube or highest serum dilution showing definite agglutination is reported as the titer.

25. Platelets can immunize a multiple-transfused patient.

26. Keeping good records makes the job easier and more orderly. It also protects the patient from simple administrative error that could harm him. It protects you, the technician, against charges of negligence in the event of transfusion problems.

27. (a) DD Form 572. Blood Donor Card.
    (b) SF 518. Blood Transfusion.
    (c) Blood bank ledger.

28. Incompatible.

29. Saline, albumin, and antihuman globulin.

30. To prevent a transfusion reaction.

31. The crossmatch procedure does not detect weakly reacting subgroups and errors in Rh typing.

32. In an emergency, at the request of the physician. He must sign a release accepting full responsibility.

33. The blood bank can add nothing to a unit of blood to be used for transfusion.

34. (a) Pyrogenic reactions.
    (b) Allergic reactions.
    (c) Embolism.
    (d) Hemolytic reactions.
    (e) Viral or bacterial infection.

35. Embolism.

36. The two most dangerous transfusion reactions are hemolytic reactions and embolisms.

37. The most often transmitted disease is hepatitis.

38. (a) Hepatitis-associated antigen (HAA).
    (b) It is a virus-like particle that measures about 20 mμ.

39. Methods for detecting hepatitis-associated antigen are:
    (a) Gel Diffusion.
    (b) Hemagglutination Inhibition.
    (c) Complement-Fixation.
    (d) Inhibition Crossover Electrophoresis (ICEP).
    (e) Counter Immunoelectrophoresis (CEP).
    (f) Radioimmunoassay (RIA).
    (g) Latex-Fixation.

CHAPTER 4

1. A good donor-technician relationship is necessary in order to encourage donors to return and donate again when they are needed. This is especially true of the first-time donor, whose initial impression may determine if he will be a repeat donor or not.

2. On DD Form 572, Blood Donor Record Card.

3. The brief physical examination given blood donors usually includes pulse, temperature, hemoglobin. weight, and blood pressure.

4. The Blood Donor Record Card documents the history of the unit of blood. If the unit becomes contaminated, the person collecting the unit can be traced so that his technique can be improved. If the patient develops a transfusion-caused infection, the donor can be traced and advised to seek medical help.

5. Fasting donors tend to feel ill more frequently than nonfasting donors.

6. (a) Serum hepatitis.
   (b) Malaria.
   (c) Tuberculosis.

7. Refer the donor to a physician for evaluation.

8. No. As long as the patient shows reaction, he is unacceptable.

9. No. The cutoff point for males is 13.5 gm. Do not collect below this value.

10. The first thing you, the collector, should do is to check the Blood Donor Record Card to be sure that the donor is eligible to give blood.

11. Alcohol is only moderately effective as an antispetic. Its main use on the phlebotomy sire is debridement, cleansing of body oils and grime.

12. To reduce cellular activity and proliferation of bacteria.

13. Plus or minus 2°C.

14. If it is connected to the regular electrical outlet, the alarm will not go off if there is a power failure.

15. (a) Ward refrigerators may not maintain the proper temperature.
    (b) The blood may be exposed to contaminating substances.
    (c) Control over the blood is lost.

16. Freezing blood.

17. The rapid freeze technique uses liquid nitrogen to freeze the blood. This is done at −197°C. and requires 60-75 seconds. The slow freeze technique uses dry ice in ether or refrigeration to obtain the required *minus* 75° to 85° C. temperature. Two or three hours are required in this method.

18. (a) Reduced acidosis.
    (b) Reduced anticoagulants are transfused.
    (c) Rare blood types can be immediately available.

19. "Human Blood" in large letters.

20. A DD Form 573 must accompany each shipment of blood in the DOD blood program. This form or a similar one should accompany each shipment to private blood banks or other facilities.

21. No. Dry ice will lower the temperature too much and the blood will freeze.

22. The military blood program is administered by the Deputy Assistant Secretary of Defense (Health and Medical). The Army's Surgeon General has direct responsibility for operating the program.

23. To the Armed Forces Whole Blood Processing Laboratory at McGuire AFB, New Jersey.

STOP— **1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.** **2. USE NUMBER 1 PENCIL.**

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VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp #1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a #1 black lead pencil.

NOTE: TEXT PAGE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Text Page Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Text Pages* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed*. Go to the VRE booklet and locate the *Text Page Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (001) Routine blood grouping and typing are performed to identify

   a. the number of X or Y chromosomes inherited.
   b. serum antigens.
   c. phenotype.
   d. genotype.

2. (001) Female germ (sex) cells have

   a. one X chromosome.
   b. two X chromosomes.
   c. both X and Y chromosomes of equal size.
   d. an X chromosome and a smaller Y chromosome.

3. (001) In hemophilic males, how many hemophilia genes are found on the X chromosome?

   a. One.
   b. Two.
   c. Twenty four.
   d. Forty eight.

4. (003) A single chromosome may carry how many genes for ABO blood groups?

   a. One.
   b. Two.
   c. Four.
   d. Six.

5. (003) Any two genes that determine the same characteristic and are located at opposing positions on matching chromosomes are

   a. loci.
   b. alleles.
   c. homozygous.
   d. heterozygous.

6. (003) Which of the following terms refers to a particular position of genes on a chromosome?

   a. Locus.
   b. Allele.
   c. Homozygous.
   d. Heterozygous.

7. (003) In the Rh system, how many genes are inherited by an offspring from each parent?

   a. One.
   b. Two.
   c. Three.
   d. Six.

8. (003) To definitely determine an individual's genotype, we must

   a. type for each Rh antigen.
   b. calculate probabilities.
   c. perform family studies.
   d. type for each Rh gene.

9. (004) Combining sites of an antibody molecule

   a. are always complementary to the whole antigenic determinant.
   b. may be complementary to only a part of the antigenic determinant.
   c. are not complementary to the whole antigenic determinant.
   d. react independently of the antigenic determinant.

10. (005-006) Initial introductions of an isoantigen into a subject produce which effect?

   a. Immediate antibody response.
   b. Immediate reaction with the body's antibodies.
   c. Delayed reaction resulting in death.
   d. Antibody response after a week or two.

11. (005-006) Which expression best describes the condition that results when a patient has been sensitized by a previous blood transfusion?

   a. Isoimmunization.
   b. Autoimmunization.
   c. Agglutination.
   d. Hemolysis.

12. (006) Which statement below best describes anti-A antibodies?

   a. Naturally occuring.
   b. Transient antibodies.
   c. Normal antibodies.
   d. Isoantibodies.

13. (006) Which of the following is *not* a property of most antibodies?

   a. Gamma globulin.
   b. Macroglobulin.
   c. Low molecular weight.
   d. High molecular weight.

14. (006) The sedimentation constant method for classifying antibodies according to their molecular weights is expressed in

   a. Svedberg units.
   b. reaction units.
   c. angstroms.
   d. moles.

15. (006) The most common subgroup of the A antigen is which of the following?

   a. A_2.
   b. A_3.
   c. Am.
   d. Ax.

16. (006) False agglutination is best described by which term below?

   a. Nonspecific clumping.
   b. Rouleaux.
   c. Prozone.
   d. Postzone.

17. (006) Serum that shows hemolysis should *not* be used in the blood bank because it might mask which of the following?

a. Antigen-antibody reactions.
b. False agglutination.
c. True agglutination.
d. Sensitization.

18. (006) Autoagglutination occurs most frequently at what temperature?

a. 56° C.
b. 37° C.
c. 20° C.
d. 5° C.

19. (007) Fibrin in a cell suspension may result in which of the following conditions?

a. Rouleaux.
b. Hemolysis.
c. False agglutination.
d. Nonspecific agglutination.

20. (007) In antigen-antibody reactions, an excess of antigen may result in

a. hemolysis.
b. a weak reaction.
c. too much reaction.
d. autoagglutination.

21. (007) Prozone is described as the inhibition of a reaction due to excess

a. antibody.
b. antigen.
c. agitation.
d. centrifugation.

22. (007) How should you correct a prozone reaction?

a. Incubate longer.
b. Centrifuge longer.
c. Dilute the antibody.
d. Dilute the cell suspension.

23. (007) The thermolabile substance responsible for hemolysis of red blood cells in antigen-antibody reaction is

a. albumin.
b. complement.
c. macroglobulin.
d. gamma globulin.

24. (008) If a baby is born with severe hemolytic disease, the only effective treatment is to perform

a. an amniocentesis procedure.
b. an exchange transfusion.
c. a normal transfusion.
d. a bilirubin test.

25. (008) If the mother is group A and the infant is group B, which type of blood is preferable for exchange transfusions?

a. A negative.
b. B negative.
c. AB negative.
d. O negative.

26. (008) The Rh_o immune globulin solution used to combat hemolytic disease of the newborn (HDN) is a solution of

a. specific substances.
b. complement.
c. antibodies.
d. antigens.

27. (009) The Rh_o immune globulin solution should be stored at what temperature?

a. Frozen.
b. 4 to 6° C.
c. 10 to 20° C.
d. Room temperature.

28. (010) The Lewis blood system is similar to which of the following other blood group systems?

a. Rh system.
b. ABO system.
c. Kell system.
d. Kidd system.

29. (011) ABO antibodies found in blood are produced as a result of

a. heredity.
b. genetics.
c. natural stimulus.
d. a prenatal immune response.

30. (011) The most common blood group in the United States is

a. O.
b. A.
c. B.
d. AB.

31. (011-012) Which of the following terms refers to the O gene having no effect on antigen precursor substances?

a. Amorph.
b. H substance.
c. Anti-H antibody.
d. Mucopolysaccharide.

32. (012) Acquired B-like antigens in a serum are probably the result of

a. old outdated cells.
b. unused gamma globulins.
c. plant lectins.
d. bacterial enzymes.

33. (012) In blood-typing, which of the following is *least* useful in detecting weak B antigens?

a. Genetic studies.
b. Anti-B typing serum.
c. Plant lectins.
d. Antibody elution.

34. (013) In serum grouping, what type of cells must often give erroneous results for subgroups of A?

a. O.
b. A_1.
c. A_2.
d. A_2 B.

35. (013) Cells suspended in saline and used for serum grouping should be discarded after

a. 1 day.
b. 3 days.
c. 10 days.
d. 21 days.

36. (013) Sensitization due to a Lewis antigen is rarely seen because normally these antibodies will *not* react

a. at low temperatures.
b. at body temperatures.
c. with complement.
d. with A or B cells.

37. (013) Which of the following types of antibodies cross the placenta and damage the fetus?

a. IgA.
b. IgG.
c. IgM.
d. IgD.

38. (013-014) D^u positive blood is commonly detected by using which of the following procedures?

a. Antibody elution.
b. Complete genotyping.
c. Direct Coombs.
d. Indirect Coombs.

39. (014) Absence of Rh antigens on a cell is thought to be due to

a. specific substances.
b. suppressor genes.
c. sex-linkage.
d. variants.

40. (014) The suspected precursor substance for Rh antigens in blood is

a. specific substances.
b. H substance.
c. Le antigens.
d. LW antigens.

41. (015) Private antigens in a serum are those that occur

a. rarely.
b. in most people.
c. very frequently.
d. in certain countries.

42. (015) Which of the following antigens is a public antigen?

a. Lewis.
b. Kell.
c. Kidd.
d. Vel.

43. (016) The size of the antibody molecule will affect our ability to

a. wash cells.
b. suspend cells.
c. centrifuge cells.
d. observe reactions.

44. (016-017) Through the use of AHG (Coombs serum), reactions are

a. enhanced.
b. reduced.
c. delayed.
d. prevented.

45. (017) Cells must be thoroughly washed before Coombs testing to remove

a. globulin.
b. complement.
c. albumin.
d. antibodies.

46. (017) Scratched glassware might cause which of the following reactions in a Coombs test?

a. False-negative.
b. False-positive.
c. Autoagglutination.
d. Rouleau.

47. (017) The qualitative presence of antibodies in serum is determined by use of

a. reference cells.
b. globulin.
c. protein.
d. hemolysin.

48. (018) When identifying antibodies, reactions in AHG, albumin, and saline tend to indicate

a. a strong antibody
b. an unusual antibody.
c. a single antibody.
d. several antibodies.

49. (018) Stronger than normal reactions that occur with specific combinations of genes are known as

a. genotype.
b. phenotype.
c. dosage.
d. heterozygous.

50. (018) Enzyme solutions used in antibody identification only work well at optimum

a. pH.
b. titer.
c. dilution.
d. concentration.

51. (018-019) The technique useful in removing unwanted antibodies from serum is

a. elution.
b. absorption.
c. hemagglutination.
d. neutralization.

52. (019) Which of the following is *not* considered when calculating titers in blood banking?

a. Volume of serum.
b. Volume of cells.
c. Volume of saline.
d. Dilution.

53. (022-023) The second copy of a set of transfusion forms (3F 518) should be marked

a. "suspense."
b. "file copy."
c. "record copy."
d. "return to laboratory."

54. (022-023) After a transfusion has been completed, the suspense copy should be

a. filed.
b. retained.
c. destroyed.
d. returned to the laboratory.

55. (022-023) Blood that has been crossmatched should be

a. released immediately.
b. released and stored on the ward.
c. released only for emergencies.
d. signed for and released as needed.

56. (024) Fresh frozen plasma should be stored at

a. −5°C.
b. −10°C.
c. −20°C.
d. −40°C.

57. (024) To correct thrombocytopenia, the patient is usually given

a. fresh plasma.
b. human albumin.
c. platelets.
d. packed cells.

58. (024) The primary purpose of the blood crossmatch is to

a. select healthy donors.
b. identify unknown antigens.
c. identify unknown antibodies.
d. prevent a transfusion reaction.

59. (024-025) A blood crossmatch will not detect Rh typing errors because Rh antibodies are

a. not naturally occurring.
b. not always present.
c. difficult to detect.
d. detectable with AHG only.

60. (025) According to AFM 168-4, blood earmarked for a pati[illegible] is to be held for

a. 12 hours.
b. 24 hours.
c. 36 hours.
d. 48 hours.

61. (027) Platelet concentrates should be used within

a. 0 to 30 minutes.
b. 1 to 2 hours.
c. 6 hours.
d. 12 hours.

62. (027) Clots in the collection bag can be prevented best by

a. adding more anticoagulant.
b. thorough mixing.
c. using heparin.
d. faster collection.

63. (027-028) Which of the following is the most frequent cause of transfusion reactions?

a. Weak reagents.
b. Atypical antibodies.
c. Contaminated blood.
d. Patient identification errors.

64. (027-028) Which of the following is the most severe form of transfusion reaction?

a. Hives.
b. Pyrogenic reaction.
c. Hemolytic reaction.
d. Chills and fever.

65. (029) The federally required method for detecting hepatitis-associated antigen (HAA) is

a. immunodiffusion.
b. complement-fixation.
c. radioimmunoassay (RIA).
d. counter immunoelectrophoresis (CEP).

66. (029) A key feature of the radioimmunoassay procedure is the antigen-antibody

a. neutralization reaction.
b. precipitation complex.
c. hemolysis reaction.
d. "sandwich" complex.

67. (030) Accidental injury to a donor having a low hemoglobin usually can be prevented if you

a. check the Donor Record Card.
b. collect the blood more slowly.
c. collect less than a full unit.
d. make a good venipuncture.

68. (030-031) Which of the following diseases most often develops as a result of giving the patient blood from an unhealthy donor?

a. Syphilis.
b. Pneumonia.
c. Influenza.
d. Hepatitis.

69. (031) Healthy civilian donors are acceptable if they are within the age bracket of

a. 12 to 15.
b. 17 to 40.
c. 21 to 60.
d. 21 to 80.

70. (031) What is the minimum period that should elapse between blood withdrawals from a donor?

a. 4 weeks.
b. 6 weeks.
c. 8 weeks.
d. 10 weeks.

71. (031) Which of the following conditions most often increases the chances of a donor reaction while giving blood?

a. Strict fasting.
b. Eating a fatty meal.
c. Drinking too much fluids.
d. Eating a nonfatty meal.

72. (031) A person with which of the following diseases should be referred to a physician for donor qualification determination?

a. High blood pressure.
b. Rheumatic fever.
c. Skin infection.
d. Asthma.

73. (031-032) Immunization with which of the following agents does *not* disqualify a donor?

a. Influenza vaccine.
b. Oral polio vaccine.
c. Diphtheria.
d. Tetanus.

74. (031-032) Immunization for rabies will disqualify a donor for

a. 6 months.
b. 2 weeks.
c. 1 week.
d. 1 year.

75. (032) A female donor should have a hemoglobin level of at least

a. 11.5 gms.
b. 12 gms.
c. 12.5 gms.
d. 14 gms.

76. (032-033) What is the maximum total volume that can be collected in the plastic blood bag used with standard blood collecting equipment?

a. 250 ml.
b. 450 ml.
c. 500 ml.
d. 600 ml.

77. (033) Plastic bags, rather than glass containers, particularly enhance the collection of

a. plasma.
b. platelets.
c. erythrocytes.
d. leukocytes.

78. (034) Recommended materials for cleaning a blood donor's arm include, in proper order, surgical soap,

a. 70 percent alcohol, and merthiolate.
b. merthiolate, and 70 percent alcohol.
c. 90 percent alcohol, and merthiolate.
d. merthiolate, and 50 percent alcohol.

79. (034) Immediately after blood enters the collection bag, the collector should

a. check the donor card.
b. agitate the bag rapidly.
c. check tube and bag numbers.
d. mix the blood and anticoagulant slowly.

80. (034) If the donor becomes violently ill while you are taking his blood, you should *first*

a. cover his forehead with a wet towel.
b. use an ammonia inhalant.
c. elevate his feet.
d. withdraw the needle.

81. (035) The primary purpose of refrigerating blood is to

a. prevent bacterial growth.
b. reduce bacterial activity.
c. reduce cellular activity.
d. maintain cellular activity.

82. (035) Blood should be stored at a temperature of 1°C. to 6°C. and this temperature should vary no more than

a. 1°C.
b. 2°C.
c. 5°C.
d. 6°C.

83. (035) With rapid freeze techniques, what medium is used to lower the temperature of blood very quickly?

a. Dry ice.
b. Helium.
c. Liquid oxygen.
d. Liquid nitrogen.

84. (036) Before cells to be deglycerolized are washed, they are diluted with

a. 10 percent glucose.
b. 30 percent glucose.
c. 50 percent glucose.
d. 80 percent glucose.

85. (036) What apparatus is used to prepare frozen blood for use?

a. Hemolyzer.
b. Aspirator.
c. Centrifuge.
d. Cytoglomerator.

86. (036) After complete deglycerolization, frozen cells are suspended in

a. fructose.
b. glucose.
c. saline.
d. ACD.

87. (037) When blood is shipped within the United States, the receiving unit should be notified of the time of arrival by

a. registered mail.
b. messenger.
c. telephone.
d. airmail.

88. (037) Blood should be shipped so that it will arrive at the receiving unit within

a. 1 day.
b. 2 days.
c. 3 days.
d. 4 days.

89. (037) During World War II and the Korean War, great improvements were made in treating battle casualties through the use of

a. blood substitutes.
b. whole blood.
c. fresh plasma.
d. frozen blood.

90. (039) After giving blood, the donor should remain in a supervised recovery area for at least

a. 15 minutes.
b. 30 minutes.
c. 45 minutes.
d. 1 hour.

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CDC 90413

MEDICAL LABORATORY TECHNICIAN-HEMATOLOGY, SEROLOGY, BLOOD BANKING, AND IMMUNOHEMATOLOGY

(AFSC 90470)

Volume 3

Serology

Proficimus More Irretenti

10-4

Extension Course Institute
Air University 195

Preface

THIS THIRD volume of CDC 90413 is concerned with clinical serology. Chapter 1 discusses concepts of the immune response and the interaction of antigen and antibody. The special reactions that produce agglutination as an end result are discussed in Chapter 2. Latex-fixation, precipitin, and antistreptolysin tests are presented in Chapter 3. A short historical review of syphilis and a discussion of various serological tests for syphilis are presented in Chapter 4. The last chapter of the volume is devoted to administrative organization of clinical laboratories in the USAF Medical Service.

A glossary of technical terms used in Volumes 1, 2, and 3 of this CDC is shown at the back of Volume 3.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to SHCS (MST/114), Sheppard AFB TX 76311.

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This volume is valued at 24 hours (8 points).

Material in this volume is technically accurate, adequate, and current as of March 1973.

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Principles of Serology

MANY DEADLY diseases have swept the world from time to time, and thousands have died as a result. But a substantial number of people have managed almost miraculously to survive these epidemics. Probably one of the earliest medical observations made by civilized man was that certain people who survived a disease were somehow protected from reinfection. This protection was usually attributed to some mystical power.

2. Today we know that our bodies produce substances that protect us against reinfection. We also know that the agent causing the disease stimulates our body to produce these protective substances. In the laboratory it is not always possible to isolate and identify the infecting microorganism, but we can usually study the body's response to infection and thereby gain an indication of the nature of the causative agent.

3. In this chapter we shall concern ourselves with an infected person's defensive mechanism against an infectious agent. This requires an understanding of natural and acquired defenses and the laboratory tests used to monitor the patient's response. Your study of immunity is an important contribution which aids the physician in diagnosis and treatment of infectious diseases.

1. Mechanics of Immunity

1-1. When a person comes into contact with microbes which cause an infectious disease, his body responds in an attempt to counteract the effect of the disease-producing agents. If the response is such as to provide partial or complete protection, then that person is said to have a certain degree of *immunity*. Immunity, which may be either natural or acquired after birth, is the subject matter of the science of immunology.

1-2. **Immunological Concepts.** We may further define immunology as "that body of knowledge concerned with the biological, chemical, and physical factors that contribute to the body's resistance to immunogenic agents." *Immunogenic agents* are those bacteria, viruses, and other substances that are capable of causing the body to produce protective substances called *antibodies*. We generally refer to these immunogenic agents as *antigens*. As we will see later in this section, antibodies have many similar properties, i.e., all are globulins. Most importantly, all antibodies at one time or another appear in the blood. If blood is allowed to clot, the serum portion can easily be obtained for immunological procedures. The studies on serum specimens that you perform in laboratory diagnosis of infectious diseases are commonly called *serological tests*. These laboratory investigations comprise that specialized field of immunological study known as *serology*.

1-3. You are probably familiar with various expressions of immunity. It has almost become a habit for us to associate immunity with infectious processes caused by bacteria, viruses, or rickettsial organisms. However, you should not lose sight of the fact that immunological responses are very often caused by agents other than living microorganisms. Many substances foreign to the body are capable of being antigenic—plant pollens for example. Usually these antigens are protein in composition, but polysaccharides, lipids, and other chemical entities may also stimulate an immune response.

1-4. **Natural Resistance.** When we speak of natural resistance to disease we are referring to immunity dependent upon some special property of a particular animal species rather than to a specific antibody. For instance, the fact that we are human gives us a certain nonsusceptibility that is different from that of the lower animals. Conversely, many animals have a natural resistance quite different from that of human beings. We know, for example, that foot-and-mouth disease rarely affects man, whereas it produces a fatal infection in cloven-hoofed animals. Tuberculosis is common in humans, cattle, pigs, and fowl, but it is uncommon in sheep, cats, dogs, and horses. Coldblooded animals such as snakes and turtles are not susceptible to gonorrhea, mumps, typhoid fever, measles, and many other important human diseases.

1-5. **Innate or Natural Immunity.** Much time and effort has been given to the study of why one individual is susceptible to a given disease but another person is not. For example, in all of the

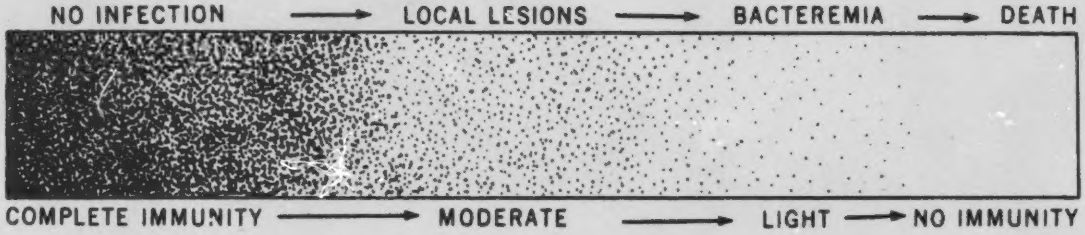


Figure 1. Levels of Immunity.

great killing epidemics that have plagued man some lucky individuals were immune and did not become ill. Also, it is a well-established fact that one's ethnic group plays an important role in determining the effectiveness of one's immunity. People living in isolated communities in Africa, on islands in the Pacific Ocean, and in Artic areas are much more susceptible to tuberculosis and smallpox than the people of Europe. Innate immunity is particularly noticeable when some members of a close group, such as a family, contract an infectious disease while other members of that same family remain disease-free. The reasons this happens or what factors operate to confer innate immunity are not perfectly known. We do know, however, that the degree of immunity is significantly influenced by the following:

a. Age.
b. Sex.
c. Diet.
d. The body's physical barriers (skin, mucous membranes, hairs lining the respiratory tract, etc.).
e. Chemical substances produced by the body (serum complement, properdin, phagocytin, lysozyme, etc.).
f. Acidity or alkalinity of digestive juices and other body fluids.

1-6. **Acquired Immunity.** This type of immunity is obtained or develops after birth. A baby derives immune substances to protect it in breast milk from its mother. Typhoid fever infection usually confers resistance to reinfection if the patient lives. In both these instances immunity is gained in the form of antibodies. In the first instance they were produced by the mother and incidentally or passively given to the baby. In the second instance the typhoid infection causes the patient's body to actively respond and produce antibodies. There are important differences between these two types of acquired immunity.

1-7. *Active acquired immunity.* Immunological protection that is acquired as a result of response to infection is called *active immunity.* In general, the presence of microorganisms and other immunogenic agents stimulates certain tissues to produce antibodies against the invader. Hence the body becomes immunized against reinfection. While some antibodies persist for many months, or even a lifetime in certain cases, some organisms, such as the *Neisseria,* are not antigenic or else produce antibodies which circulate in the blood stream for only a short period.

1-8. Active immunity is not always perfect and may be graded into a series of levels that extend from complete immunity to a state approaching complete susceptibility. Since the body's defenses can often be overcome if the challenging dose of microbes is large enough, several grades of illness may occur between the two extremes. Figure 1 illustrates the levels of immunity. In this illustration resistance becomes progressively lower as you move from a high level of immunity (heavy stippling) to an absence of immunity (no stippling).

1-9. *Passive acquired immunity.* The second type of acquired immunity is *passive immunity.* In this type of immunity, antibodies from the blood of one animal or person are injected or transferred to give immunity to a second organism. The body producing the antibodies is not necessarily of the same species as the animal that receives the protection. Thus, antibodies produced as a result of injecting horses, rabbits, and other animals with antigens are routinely purified, concentrated, and injected into humans to protect against such diseases as tetanus, diphtheria, typhoid fever, and botulism. In the cases above, the solution injected into the human is referred to as antitoxin, but it is a solution of antibodies, nonetheless. (Antitoxins are antibodies against a toxin.)

1-10. The immunity gained by passive immunization is usually temporary. It protects immediately after injection and for a short time thereafter. The procedure is particularly useful in providing protection in the critical periods of infancy and early childhood. Passive immunizing agents are not usually thought of as being themselves immunogenic. That is, they do not elicit antibody response against the infectious agent they are given to protect against. However, since they are produced in other animals, injections of serums from these animals can result in sensitization. For example, frequent injections of horse serum will cause sensitization and result in serum sickness.

2. Antigen-Antibody Interaction

2-1. Much is known today about antigens, antibodies, and their reactions. Scientific study of these entities began in the latter part of the 19th century. While most of the early work concerned bacteria and their antigens, later research has taken great strides forward in blood transfusion technology. For the most part, these fields of study were separate endeavors for many years. Consequently, the immunohematologist developed definitions quite different from those used by the bacteriologist. Only recently have we begun to understand that serological reactions encountered in the two fields are expressions of a fundamental occurrence—the antigen-antibody reaction.

2-2. **Antigens.** To understand why antigens react as they do, we must first define what they are and then discuss their unique properties. The word *antigen* is derived from the Greek words *anti* (against) and *gennen* (to produce). In today's terms we mean a substance which induces the formation of antibodies when introduced into an animal and then reacts with these antibodies. The reaction is usually observable in some way in the laboratory.

2-3. Historically, the most often studied and best known antigens were *proteins*, especially bacterial and blood cell proteins. Quite a few other substances make good antigens. Foremost among these are the *polysaccharides*. Polysaccharides are found quite extensively in nature, particularly as a coating or capsule on bacteria. They are unusual in that they may stimulate antibody production in one kind of animal, i.e., mice, but not in others, such as rabbits or guinea pigs. Individual capsular polysaccharides may differ a bit from one another. In fact, the well-known capsular polysaccharides of the pneumococcus have been divided into 80 different types, each of which, if injected into an animal, causes it to form antibodies specific for that type alone. Thus we have 80 different types of antibodies for pneumococcal polysaccharides.

2-4. Some other substances that are antigenic include:

a. Lipopolysaccharides—found in many microorganisms, especially the clinically important gram-negative Enterobacteriaceae such as *Salmonella* and *Shigella* species.

b. Glycoproteins and glycopeptides—the blood Group A and B substances found in mucosal cell secretions of some people are glycopeptides.

c. Polypeptides—gelatin is an example.

d. Nucleic acids—certain types of deoxyribonucleic acid (DNA) are antigenic. People with lupus erythematosus possess antibodies against a particular form of DNA.

e. Low molecular weight substances—this group includes cardiolipin such as that used in syphilis serology.

2-5. Immunogenic substances have many characteristics, both physical and chemical, that are common to them as a group. Some of these characteristics are:

a. The antigens, except in rare instances, must be foreign to the body. "Foreign" here means foreign to the individual's antibody forming tissues.

b. Antigens usually possess molecular weights of 10,000 or higher.

c. Antigens stimulate the formation of specific antibodies. These antibodies must react with the antigen in some observable way.

2-6. For some unknown reason, some individuals with degenerative tissue diseases produce antibodies against abnormal tissue within their own body. These diseases are called *autoimmune diseases* and are thought to occur because the degenerative abnormal tissue is more or less foreign to the person's body and is therefore capable of being immunogenic. *Hashimoto's Disease* (thyroiditis) is the most often cited example of this condition. Other diseases of the thyroid (goiter, hypothyroidism) have also caused the appearance of autoimmune bodies. The antibodies have been detected by precipitation, gel diffusion, hemagglutination, and immunofluorescence studies.

2-7. **Antibodies.** Antibodies are also complex proteins. This fact is stressed here because it is so often emphasized that antigens are protein in nature, and we tend to dismiss the fact that antibodies are proteins too. Antibodies are found in the globulin fraction of serum protein, but they also occur in other body fluids and tissues. Related proteins for which antibody activity has not as yet been demonstrated include Bence-Jones proteins, myeloma proteins, and others. *Immunoproteins, immunoglobulins,* and *immune bodies* are terms often used instead of *antibody*. Remember, however, that all antibodies are globulins, but not all globulins are antibodies.

2-8. Several systems have been devised to classify the various types of antibodies. These systems are based on the chemical, physical, and biological differences in antibodies. The best system uses the type of reaction between an antigen and its specific antibody as a means of classification. Thus, we have such classes of antibodies as follows:

a. Antitoxins—counteract bacterial toxins.

b. Agglutinins—cause foreign cells to clump.

c. Precipitins—render soluble foreign proteins insoluble.

d. Lysins—dissolve foreign cells.

e. Opsonins—increase the susceptibility of bacteria to phagocytosis.

f. Reagins—produce flocculation in complement-fixation and related tests.

g. Neutralizing antibodies—render the immunogenic agent (commonly viruses) harmless.

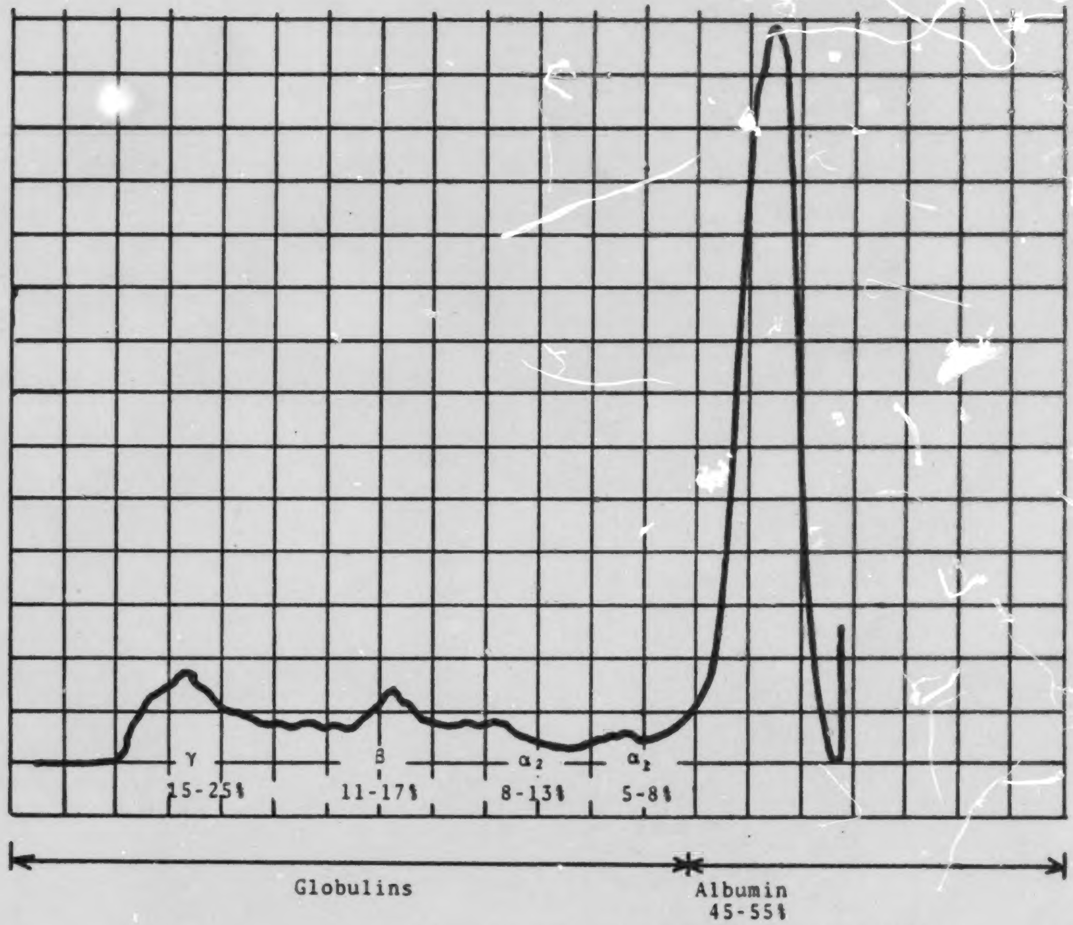


Figure 2. Human serum electrophoretic pattern.

h. Bactericidins—kill bacteria.

i. Immobilizing antibodies—stop movement of microorganisms by affecting their locomotion.

2-9. As laboratory procedures for studying proteins have advanced, other systems of classification have come into use. These systems are related to the physical characteristics of immunoglobulins—namely, their sedimentation and electrophoretic properties. We can separate serum protein into its components using the electrophoretic mobility of these components. Figure 2 shows a typical electrophoretic pattern of human serum. The two major components on this pattern are albumin and globulin. The globulins, which are of interest to us because of their immunological properties, can be further divided into three groups. These are alpha (α), beta (β), and gamma (γ) globulins. If the albumin and globulins are separated immunoelectrophoretically, fractions will separate in the order shown in table 1. These fractions can be eluted from agar gels or other media and tested for antibody activity.

2-10. Immunoglobulins can also be separated in a procedure that takes advantage of the fact that different classes of antibodies have different

Table 1
Human serum components

Globulins

Albumin: α_1^0 α_1^1 α_1^2 α_1^3: α_2^1 α_2^2: β^1 β^2 β^3 β^4 : γ γ_1 γ_2 γ_3 γ_4 γ_5: Specimen Applied Here

Direction of Migration

molecular weights. In general, immunoglobulins have very high molecular weights (125,000 to 900,000); however, if macroglobulins are included, the figure is close to 1,000,000. Separation is accomplished in an ultracentrifuge at speeds of up to 70,000 r.p.m. Solutions are placed in a quartz cell and are centrifuged. While the machine revolves, a photoelectric cell measures the density of the solution. The heaviest molecules of course sediment first, followed in order by increasingly lighter molecules. Thus the globulin solution is separated into bands containing molecules of varying densities. These bands are identified by a numeral followed by the letter S (2S, 7S, 19S, 20S, etc.). The S refers to the *sedimentation constant* or Svedberg (S) units. Analyses of globulins have shown that 7S fractions have a molecular weight of about 160,000 while 19S fractions weigh in at 900,000.

2-11. Both the electrophoretic and sedimentation constant methods of classifying immunoglobulins have been widely used. To provide a basis for comparison, a recent conference of international immunologists recommended certain standardized terms in reference to immunoglobulins and their properties. Their main recommendation is that the accepted abbreviation for immunoglobulin should be *Ig*. They recommended further that *Ig* be subdivided into fractions, e.g., *IgG (gamma), IgM (mu)* and *IgA (alpha)*. These three fractions are composed of components identified by electrophoretic mobility and sedimentation constants.

2-12. Structurally the immunoglobulin molecule is made of two and possibly three types of polypeptide molecular chains, each of which is formed genetically independent of the other. The type and amount of a particular chain present in an antibody determines its properties and reactivity.

2-13. Other differentiating factors that help separate the three Ig fractions are:

- Only IgG molecules are small enough to cross the human placenta.
- IgM is usually produced rapidly after immunization but diminishes as a strong IgG response takes over.
- IgA occurs in large amounts in tears, colostrum, saliva, and internal secretions and may provide protection where other types do not occur.

2-14. Now that we know some of the properties and something about antibody structure we can direct our attention to the site of antibody production in the body and the cells or tissues involved. Recent studies indicate that lymphoid tissue is the primary source of antibodies. Lymphocytes and plasmocytes are thought to be the cells directly involved in antibody production. As much as 60 to 70 percent of the total antibody yield may be due to lymphocytes and plasma cells. The

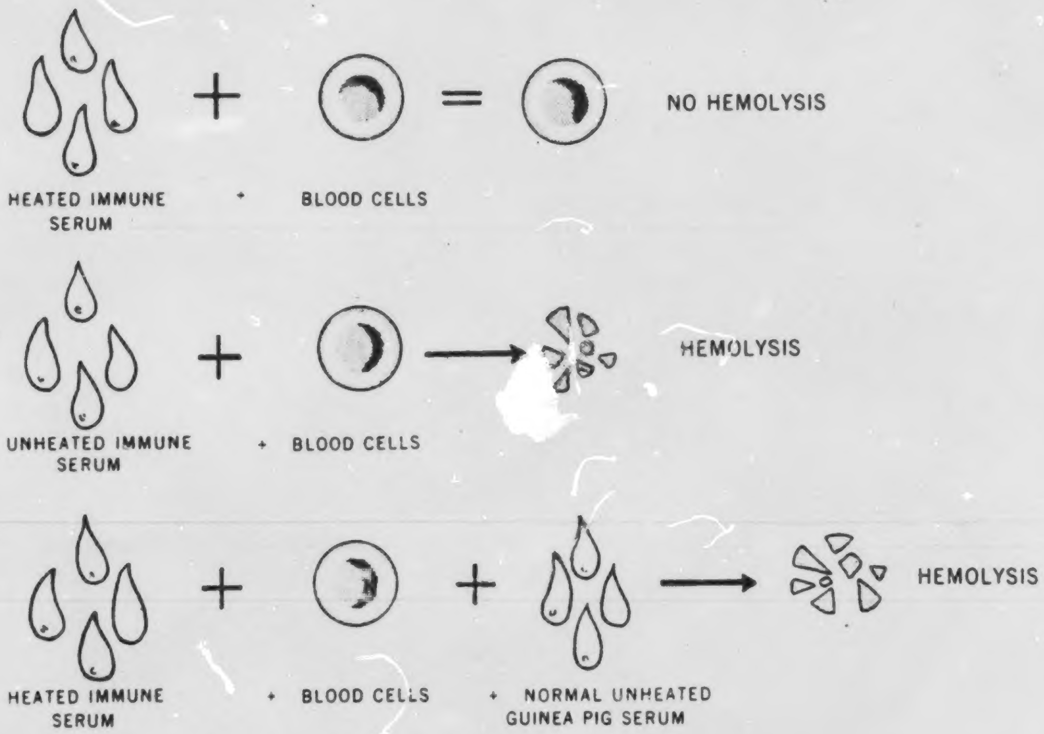


Figure 3. Lytic qualities of complement.

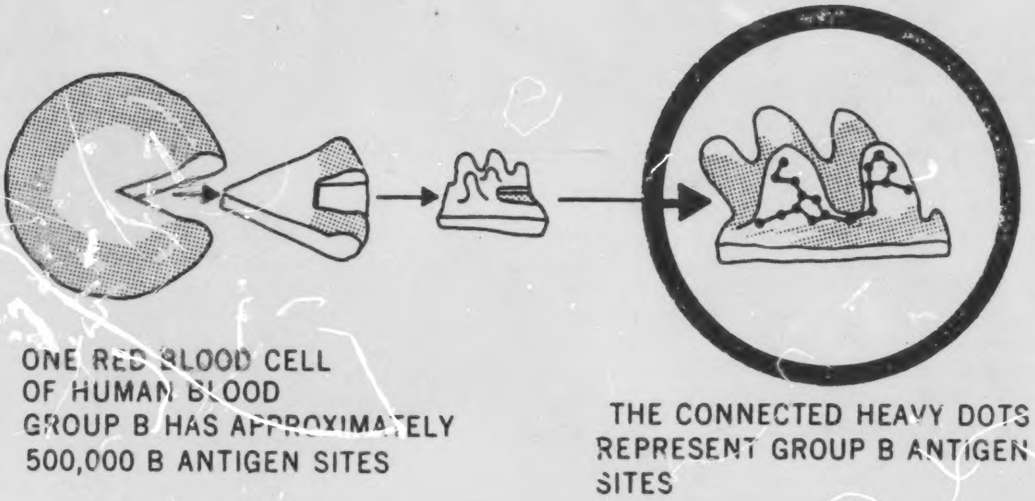


Figure 4. Schematic representation of group B antigen sites on a human red blood cell.

remaining antibody production is probably from cells such as those lining the intestinal tract and large macrophage cells. Since both lymphocytes and plasma cells develop from cells of the *reticuloendothelial system*, we may say that this system produces cells which later produce antibodies. Any cell capable of forming an antibody is called an *immunocompetent cell.*

2-15. Several theories have been advanced as to how antibodies are produced by cells. The most universal are the template theories. The *direct template* theory holds that when a foreign protein comes into contact with normal globulin as the globulin is being formed, the antigen acts as a mold which causes the newly formed globulin to assume a "mirror image" of the antigen. The antigen is like a rubber stamp—stamping out reverse identical images of itself. The *indirect template* theory states that the presence of antigen within the cell affects the genetic memory of the cell so that the cell produces an altered globulin which is passed on to succeeding generations. The *natural template* theory says that preexisting antibody templates are located in clones of mesenchymal cells. (Clones are an aggregate of cells all of which are descended from a single parent cell.) The clones react with antigens selectively; that is, they react only with certain antigens. When clones and antigens react, cells in the clone mature as plasma cells which produce an antibody specific for the reacting antigen.

2-16. **Complement.** No discussion of antigen-antibody interaction is complete without touching upon the role of complement in these reactions. Early bacteriologists noticed that certain bloods would kill pathogenic bacteria when these bloods and bacterial cells were mixed. Only certain kinds of bacteria were destroyed, and the power to destroy was present only in blood of animals immune to that particular type of bacterium. This lytic substance was found in serum as well as in whole blood. This lethal property was destroyed if the blood was heated to about 55° C. for a little less than an hour. The *Pfeiffer reaction* demonstrated that if a test serum was heated to destroy this factor and then mixed with a small amount of normal guinea pig serum, the test serum would again destroy the microorganisms. The conclusion was drawn that the lytic substance was sensitive to heat and that some other heat resistant component (antibodies) in serum is also necessary for the reaction to take place.

2-17. Bacteriolysis was not the only type of reaction this factor was responsible for. It was further demonstrated that this same lytic substance (now known as complement) would cause hemolysis when blood cells and the lytic substance were mixed with an antiserum prepared against the cells. Cytolysis or cell destruction of both bacteria and blood cells is the direct result of an antigen-antibody reaction in the presence of the lytic factor, complement. The historical observations on the lytic properties of complement are demonstrated in figure 3.

2-18. Complement occurs in the serum of most animals. The guinea pig is the usual source of complement for testing purposes because guinea pigs yield complement that is more uniform and reliable than the product from most other animals. In order to standardize our serological procedures as much as possible, complement from a single species is necessary.

2-19. When antigens and antibodies react in the presence of complement, the complement is actually bound or fixed. It is not available for reaction if other antigens or antibodies are later added to the reaction mixture. This binding effect is called *complement-fixation*. Complement becomes fixed not only in bacteriolytic and hemolytic reactions but

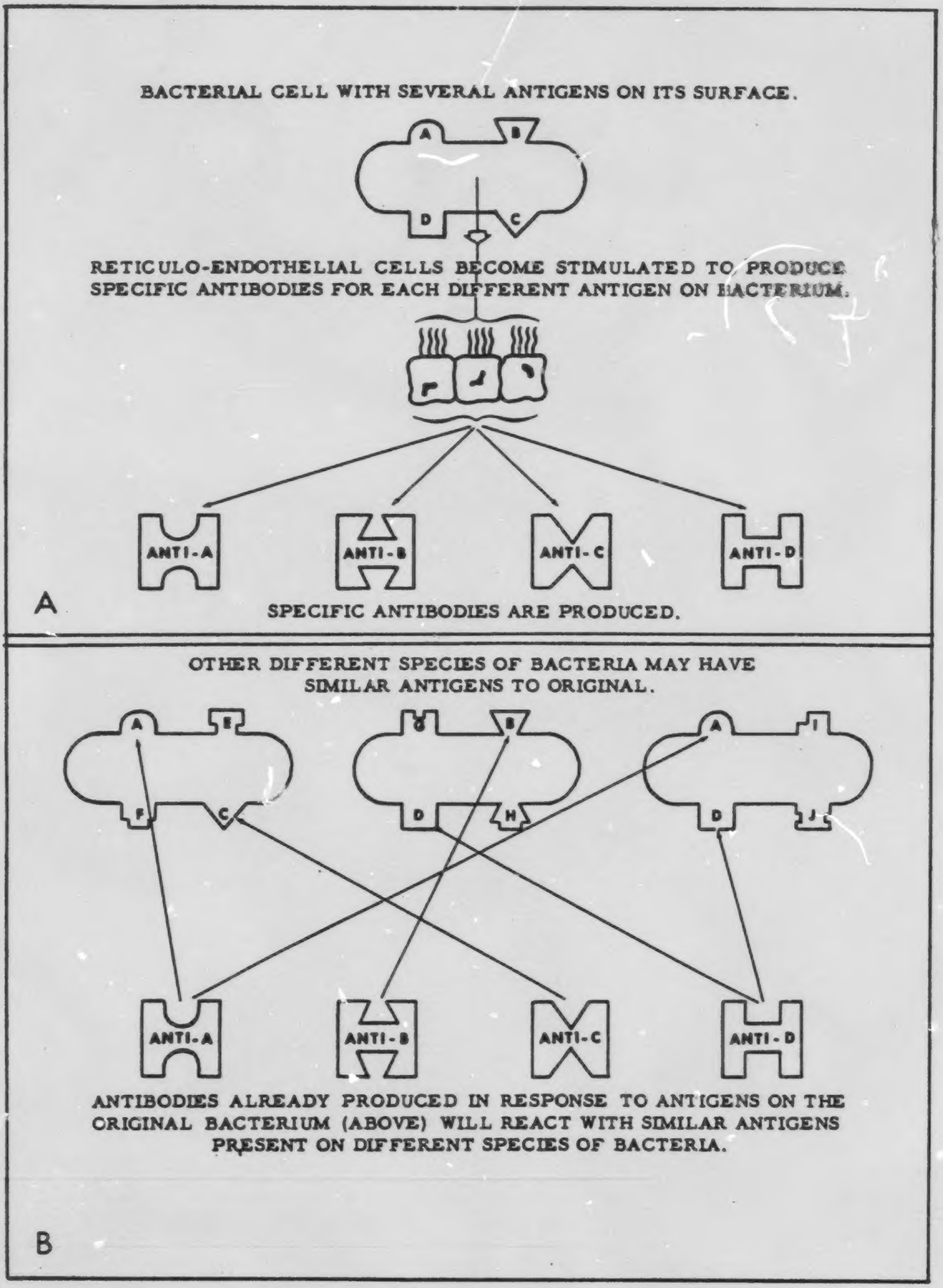

BACTERIAL CELL WITH SEVERAL ANTIGENS ON ITS SURFACE.
A
B
D
C
RETICULO-ENDOTHELIAL CELLS BECOME STIMULATED TO PRODUCE SPECIFIC ANTIBODIES FOR EACH DIFFERENT ANTIGEN ON BACTERIUM.
ANTI-A
ANTI-B
ANTI-C
ANTI-D
A
SPECIFIC ANTIBODIES ARE PRODUCED.
OTHER DIFFERENT SPECIES OF BACTERIA MAY HAVE SIMILAR ANTIGENS TO ORIGINAL.
A
E
F
C
G
B
D
H
A
I
D
J
ANTI-A
ANTI-B
ANTI-C
ANTI-D
ANTIBODIES ALREADY PRODUCED IN RESPONSE TO ANTIGENS ON THE ORIGINAL BACTERIUM (ABOVE) WILL REACT WITH SIMILAR ANTIGENS PRESENT ON DIFFERENT SPECIES OF BACTERIA.
B

Figure 5(A). Cross-reactivity.

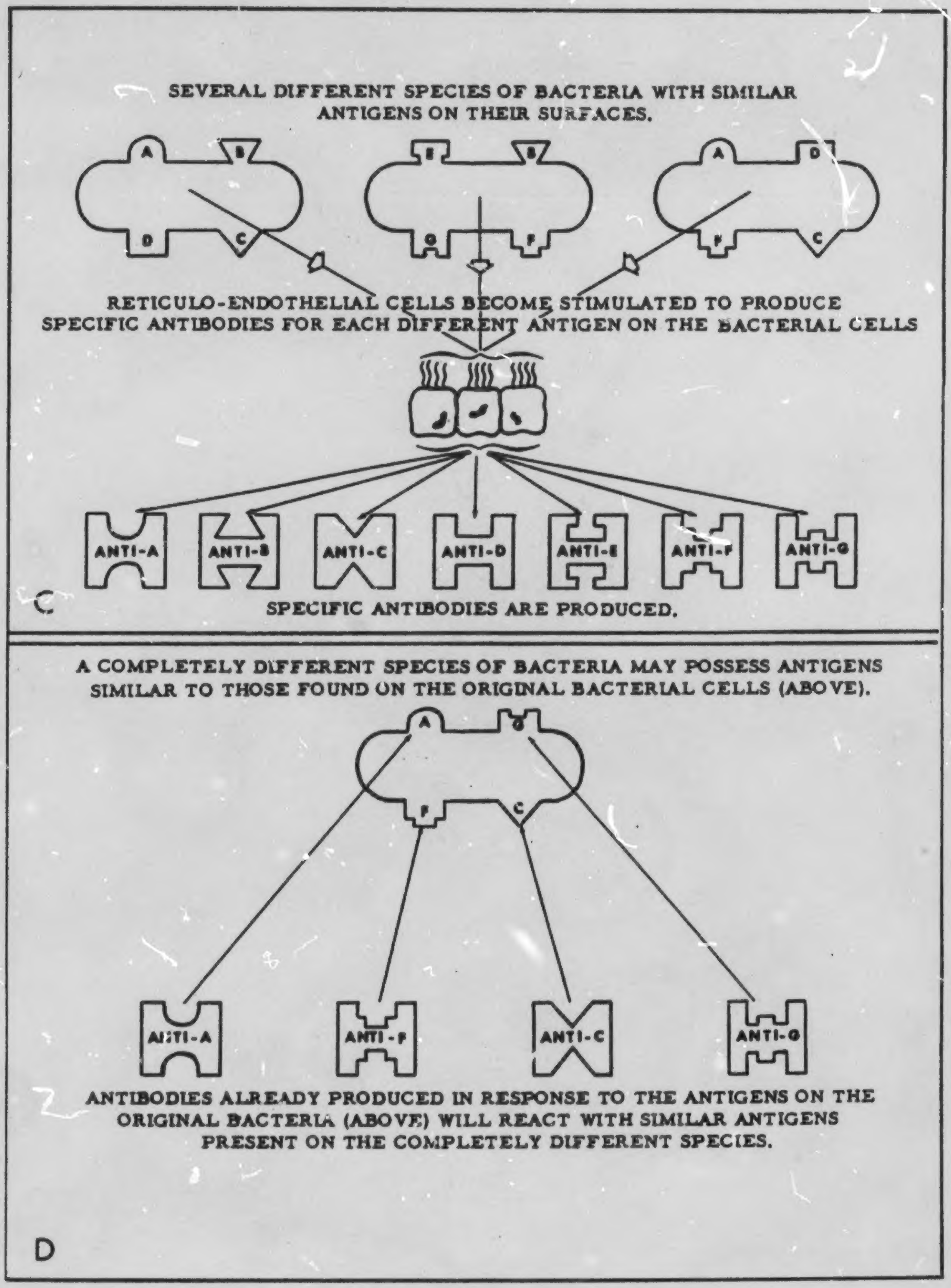

SEVERAL DIFFERENT SPECIES OF BACTERIA WITH SIMILAR ANTIGENS ON THEIR SURFACES.
A
B
D
C
E
B
G
F
A
D
F
C
RETICULO-ENDOTHELIAL CELLS BECOME STIMULATED TO PRODUCE SPECIFIC ANTIBODIES FOR EACH DIFFERENT ANTIGEN ON THE BACTERIAL CELLS
ANTI-A
ANTI-B
ANTI-C
ANTI-D
ANTI-E
ANTI-F
ANTI-G
C
SPECIFIC ANTIBODIES ARE PRODUCED.
A COMPLETELY DIFFERENT SPECIES OF BACTERIA MAY POSSESS ANTIGENS SIMILAR TO THOSE FOUND ON THE ORIGINAL BACTERIAL CELLS (ABOVE).
A
G
F
C
ANTI-A
ANTI-F
ANTI-C
ANTI-G
ANTIBODIES ALREADY PRODUCED IN RESPONSE TO THE ANTIGENS ON THE ORIGINAL BACTERIA (ABOVE) WILL REACT WITH SIMILAR ANTIGENS PRESENT ON THE COMPLETELY DIFFERENT SPECIES.
D

Figure 5(B). Cross-reactivity (cont'd).

other antigen-antibody reactions as well. Some of these reactions do not give macroscopically observable results. Nevertheless, the reaction takes place and may be demonstrated using blood cells sensitized with an appropriate antiserum as an indicator system.

2-20. Some of the activities that complement participates in are the following:

- Lethal action against certain bacteria in the presence of an immune serum.
- Lysis of bacteria in the presence of an immune serum.
- Hemolysis of blood cells sensitized with an antiserum.
- Opsonization of certain bacteria, i.e., increasing the susceptibility of bacteria to phagocytosis.

2-21. Experimentally, complement has been shown to be composed of several distinct globulin and globulinlike components. These components are labeled C'1, C'2, C'3, etc., and this labeling denotes that they are fractions of whole complement (C). Each fraction possesses qualities which allow its separation from other fractions. We will have more to say about complement in our discussion of specific complement-fixation tests in syphilis serology.

2-22. **Immunological Reactions.** The science of serology deals primarily with the detection of antibodies present in the body fluids. These antibodies are produced as a specific defense mechanism against certain substances, i.e., antigens (immunogenic agents). By demonstrating the presence of a specific antibody *in vitro*, clinical serology supplies indirect evidence of the immunogenic agent which stimulated the antibody formation.

2-23. The reaction which occurs between antigen and antibody is only partially understood. It is, however, an extremely important one for the clinical laboratory. It is thought that antigens and antibodies react because of their *complementarity*. We may compare complementarity to a lock and key concept. The lock (antigen) has tumblers (determinants) that have specific dimensions. The key (antibody) has grooves (combining sites) that fit exactly into the tumblers of the lock. When the key is inserted and turned, the door opens; when the antigen and antibody combine, a reaction takes place.

2-24. Specifically, certain atoms or groups of atoms on the antigen combine with complementary atoms on the antibody. The binding forces between antigens and antibodies are generally weak, so the antigen and antibody must be brought close together before reaction occurs. Figure 4 shows a schematic representation of determinants of an antigen on a red cell. In this representation the large black circles represent atoms or groups of atoms. Reactions take place at a single site or may involve several sites. In some reactions the molecular binding force may be fairly strong, even irreversible or unalterable. In others it may be weak and reversible.

2-25. Antigen-antibody reactions involve *specificity*. That is, antigenic determinants ordinarily react with antibody combining sites *only* if they are complementary. In some instances, though, two antigenic determinants are so similar structurally that they react with a common antibody combining site. When this occurs, we speak of it as *cross-reactivity*. Cross-reacting antigens frequently make serological tests difficult to interpret, particularly among those viruses and bacteria which share a common antigen.

2-26. Cross-reaction between an antibody and several different antigens occurs mainly among antigens that are polysaccharide in nature. Many medically important microorganisms have polysaccharide substances as part of their chemical and physiologic makeup. This substance may be either a somatic or capsular antigen. The illustrations in figures 5(A) and 5(B) demonstrate ways in which cross-reaction may occur.

2-27. Cross-reactivity may be helpful as well as a handicap. For instance, in *Salmonella* typing, a cross-reaction between species would be a handicap to precise identification. However, if we wish to screen, as in febrile agglutination tests, it becomes helpful in reducing the number of tests we have to perform. The cross-reaction between polysaccharide from certain strains of *Proteus* organisms and antibodies against certain *Rickettsia* is called the Weil-Felix reaction. This cross-reaction is useful in diagnostic tests for rickettsial diseases.

2-28. In antigen-antibody reactions an optimum amount of [illegible] component is required for the most complete [illegible]. In hemagglutination procedures agglutination is achieved by reacting varying quantities of antibodies (dilutions) with a constant volume of antigen. In precipitin reactions the usual method is to react varying quantities of antigen with a constant quantity of antibody. When an optimum amount of antigen and antibody is present in a reaction, we refer to it as an *equivalence zone*. If excess antibody is detectable in the supernatant after the reaction is over, the reaction has taken place in a *zone of antibody excess*. When there is too much antigen or antibody present for ideal reaction, falsely negative tests may occur. Traditionally, the zone of antibody excess has been referred to as the *prezone or prozone*. The zone of antigen excess is called the *postzone*.

3. Serologic Methods

3-1. Serological tests are among the oldest diagnostic tests performed in the laboratory. It has only been recently, however, that we have begun to understand the true nature of microbial and other antigens and their corresponding antibodies. With greater insight, modern serological procedures have provided the immunologist with tools to measure their presence and properties.

3-2. **Titer.** Antibody is most often measured by making several dilutions of the antibody specimen (usually serum) and allowing it to react with a constant volume of antigen. If the reaction is visible or observable we will see the reaction immediately, or within a very few minutes. Sometimes an *indicator system*, usually a cell suspension, must be added to the mixture to make the reaction visible. When reporting results, the expression *titer* is used. Titer is defined as the concentration of antibodies in serum expressed as the reciprocal of the highest dilution giving complete agglutination or maximum precipitation. Titer expresses the units or parts present in a total volume.

3-3. Titers are more accurately performed in tube procedures than in slide procedures. Recent improvements in purified antigens have allowed us to do fairly accurate rapid slide procedures. In fact, in some antigen-antibody mixtures, the reaction is much easier to read on a slide than in a tube (pregnancy test, R-A test, etc.). Titering is a simple procedure and is routinely used to quantitate the amount of antibody present in a serum specimen.

3-4. **Cell Suspensions.** Cells from various sources may be used as an antigen source or as an indicator system in serologic tests. The type of cells selected depends on the type of antigen they carry and the type of antibody we want to isolate or quantitate. Cells from humans, cows, sheep, chickens, guinea pigs, horses, and several other animals are routinely used in one serologic test or another. Usually a suspension of these cells is prepared in saline or albumin. Other media may be required, depending on the type of antibody involved.

3-5. The mathematics used in calculating cell suspensions in very simple. The following three formulas will solve any cell suspension problem.

- To find the packed cell volume when the total volume and amount of cells are known:

$$\text{Packed cell volume} = \frac{\text{total volume} \times \text{\% solution desired}}{100}$$

- To find the percent solution when the total volume and amount of cells are known:

$$\text{\% solution} = \frac{\text{packed cell volume} \times 100}{\text{total volume}}$$

- To find total volume when final concentration and amount of available cells are known:

$$\text{Total volume} = \frac{\text{packed cell volume} \times 100}{\text{\% solution desired}}$$

3-6. Problem Situation #1: To make up 75 ml. of a 3-percent suspension of sheep blood cells, what volume of packed cells is required?

3-7. Problem Situation #2: If you have 4.4 ml. of packed cells after washing and the procedure requires that you add a sufficient quantity of saline to make 100 ml. total volume, what percent cell suspension will you have?

3-8. Problem Situation #3: You have 0.36 ml. of cells after washing and you want to make a 3-percent solution. What volume of cell suspension can you prepare?

3-9. Solution to Problem Situation #1: Use the formula for finding PCV and substitute known values into the formula.

$$\text{Packed cell volume} = \frac{\text{total volume} \times \text{\% solution desired}}{100} = \frac{75 \times 3}{100} = \frac{225}{100} = 2.25 \text{ ml.}$$

q.s. 2.25 ml. PCV to 75 ml. with diluent.

3-10. Solution to Problem Situation #2: The second formula applies here.

$$\text{\% cell suspension} = \frac{\text{PCV} \times 100}{\text{total volume}} = \frac{4.4 \times 100}{100} = 4.4\%$$

This is a very hypothetical situation and probably is never encountered; however, the calculation will help you understand cell suspensions.

3-11. Solution to Problem Situation #3: Apply the last formula, the one that finds an unknown total volume, in this instance.

$$\text{Total volume} = \frac{\text{PCV} \times 100}{\text{\% solution desired}} = \frac{0.36 \times 100}{3} = \frac{36}{3} = 12 \text{ ml. total volume}$$

3-12. Be careful in preparing cell suspensions. don't cut cell washing techniques short or alter them. Skimping in any area leads to false results. Use only fresh cells, because metabolic changes in aged cells may affect test results. Old cells also require more effort in washing because they are easily hemolyzed. (When mixing cell suspensions always use paraffin film or a stopper to cover the tube opening, never your finger.)

3-13. **Serial Dilutions.** In diluting, a solution of higher concentration is made into one of lesser concentration. If this is done in a mathematical progression, e.g., reducing the concentration by half each time, we have a "serially diluted" specimen. When we reduce the concentration by half we are performing a *twofold* serial dilution. In a serial

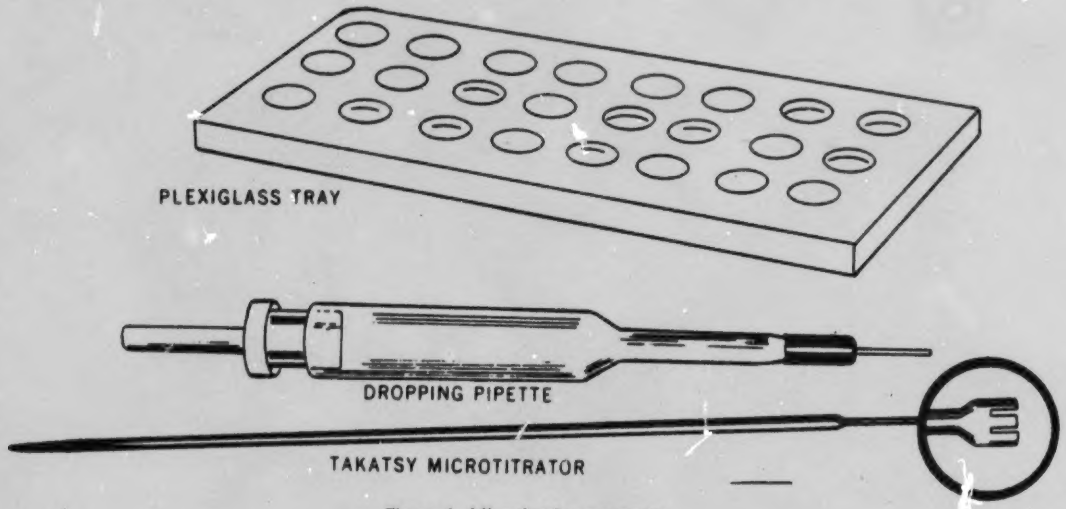


Figure 6. Microtitration apparatus.

dilution the dilution is inversely proportional to the concentration of the substance being diluted. Consequently, our dilution technique must be standardized so that when we say 1 part serum in a 1 in 10 dilution, everyone will understand the meaning. This statement means we have a total of 10 parts; 1 part is solute and 9 parts are diluent. A 1 in 10 dilution should *not* be interpreted as 1 part solute to 10 parts diluent, or a total of 11 parts. The dilution may be written as 1/10 or 1:10. If the dilution is a test result expressed as a titer, it can be written as 1/10, 1:10, or as "a titer of 10."

3-14. You will usually perform serial dilutions in test tubes. However, they can also be performed directly on a ringed slide or a slide with depressions such as a Boerner slide. One of the newer techniques for performing serial dilutions is the microtitration method originally introduced by Takatsy and modified by Sever. This method involves several simple pieces of equipment that may be obtained commercially. The basic components are a Plexiglas sheet and a calibrated loop. The Plexiglas sheet is drilled with a series of wells. U-shaped wells are used for complement-fixation tests and V-shaped cups for hemagglutination tests. Calibrated dropping pipettes are used to deliver the specimen to be tested and other reagents involved. This equipment is shown in figure 6.

3-15. The correct amount of diluent and specimen are delivered to the appropriate cups with dropping pipettes. The dilution is made by transferring a loopful from one cup to another, mixing, and transferring a loopful. This is a very rapid technique and cuts down tremendously on time and equipment required to perform a large number of seriological procedures. The loops are cleaned by flame and the Plexiglas plate is easily washed. Repeat tests or new tests can be quickly started and completed. Those laboratories performing complement-fixation, hemagglutination, and similar procedures would do well to consider this technique.

3-16. The simplest method of calculating serial dilutions is to use the following formula:

$$\text{Dilution} = \frac{\text{total volume in tube}}{\text{volume of serum in tube}}$$

The answer is always the reciprocal of the dilution. Consider the following problems which demonstrate this formula.

3-17. Problem Situation #4: You are setting up a test and you need to know the dilution of the specimen that will give maximum results. Start from table 2. What is the dilution in the first tube? Continue to calculate the dilutions through tube 7.

3-18. Problem Situation #5: You have solved the problem down to tube 7 and have obtained the results shown in table 3. Calculate the dilution of the 8th tube.

3-19. Problem Situation #6: Suppose you wanted to make a one-tube dilution with the following proportions.

Total volume = 7 ml.
Volume serum added = 0.03 ml.

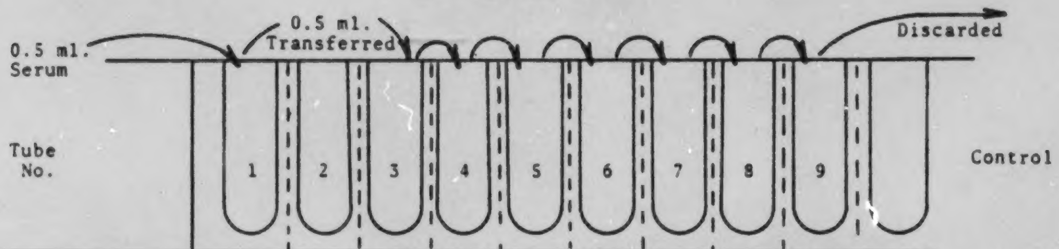
What is the dilution of the serum?

3-20. Solution to Problem Situation #4: In this instance, you can use the formula directly and solve the problem as follows:

$$\text{Total volume in tube} = (0.5 + 0.5) - 0.5 = 0.5$$

$$\text{Dilution} = \frac{0.5}{0.25} = 2 \text{ or } 1{:}2$$

Table 2
Serial dilution schematic



	1	2	3	4	5	6	7	8	9	Control
Volume Diluent	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vol. Serum After Transfer	0.25									
Dilution										

3-21. Solution to Problem Situation #5: Use the formula to solve the problem as follows:

Total volume = (diluent + diluted specimen) – transferred volume
= 0.5 + 0.5 – 0.5

$$\text{Dilution} = \frac{0.5}{0.0019} = 256 \text{ or } 1:256.$$

3-22. Solution to Problem Situation #6: This problem is solved in a similar manner.

$$\text{Dilution} = \frac{\text{Total volume}}{\text{volume serum in tube}} = \frac{7.0}{0.03} = 233.33$$
$$= 1:233.33$$

3-23. Generally, once you find the dilution of the first tube the others are easy. You should remember that titers or dilutions are sometimes figured differently for different kinds of tests. In immunohematological or blood banking tests the dilution or titer is usually figured prior to adding the cell suspension. On the other hand, in serological procedures the dilution or titer is usually calculated after the cell suspensions, hemolysin, complement, etc., are added. In the first instance the titer is based on the initial dilution of the serum. In the latter, the titer is based on the final dilution in the tube.

3-24. **Some Common Serological Techniques.** The following general methods are used in serology. Some of them will be discussed in later chapters when we consider more specific serological tests.

a. Complement-fixation test. This procedure was described in principle in CDC 90412, when we discussed virology. The test uses an indicator system of sheep red blood cells and hemolysins (antibodies against sheep RBC) to detect fixation of complement by antibodies in a patient's serum.

b. Agglutination tests. Tests in which large bodies such as blood cells or bacteria clump in the presence of certain specific antibodies.

c. Hemagglutination-inhibition tests. Tests in which the agglutination of cellular antigen is inhibited by exposing the antigen to specific competing antibodies.

d. Precipitin tests. In these tests the antigen-antibody reaction produces a visible precipitate, a flaky sediment, or soluble antigen-antibody complexes.

e. Neutralization. In this test, antigenic agents or their products are reacted with antibodies prior to injection into test animals. The absence of an effect on animals indicates the presence of antibody in the injected solution.

f. Immobilization tests. Known motile microorganisms are mixed with serum containing antibodies against some antigen of the organism. If the serum stops the motility of the microorganism, the reaction indicates the presence of specific antibodies.

Table 3
Serial dilution results

	1	2	3	4	5	6	7	8
Vol. Serum after trans	0.25	0.125	0.0625	0.03125	0.01563	0.0078	0.0039	
Dilution	2	4	8	16	32	64	128	

g. Immunodiffusion tests. An example of this test was also given in the aforementioned discussion on virology. Antigens and antibodies are allowed to react in an agar gel medium. Lines of precipitation in the gel between the antigen and its specific antibody indicate specificity. The test may also be combined with electrophoresis when the gel is applied on a microscope slide or Mylar plastic film. When this is done, specific globulin fractions involved in the reaction can be separated.

3-25. *Fluorescent antibody techniques.* Information in the remainder of this chapter is condensed from "Fluorescent Antibody Techniques," U.S. Department of Health, Education, and Welfare; Public Health Service, 1966. As you will recall from the earlier discussion, antigens and antibodies can be studied after treatment with a fluorescent dye such as fluorescein. Procedures utilizing this technique have general applications as diagnostic tools, as well as experimental applications for investigating complex antigen-antibody reactions.

3-26. A substance is said to be fluorescent if, upon absorbing a light energy of one wavelength ("excitation" or "activation" light), it emits light of another wavelength within less than 10 seconds. If the absorbed light is emitted over a longer period, the substance is called phosphorescent. Both conditions are special cases of the general phenomenon of luminescence.

3-27. The wavelength of the fluorescent light is independent of the wavelength of the light absorbed except that, due to energy losses involved, the emitted light is always of longer wavelength than the exciting light. The dye fluorescein, for example, fluoresces in the yellow-green region regardless of whether it is irradiated by ultraviolet or by blue light. The color actually seen in the fluorescence microscope depends upon the eyepiece filter employed.

3-28. Fluorescent microscopy is more demanding than ordinary "white" light microscopy because mechanical and optical alignment become more critical when the image is relatively dim. Since the brightest fluorescent field is likely to be several times less bright than the ordinary microscope field illuminated with white light, it is important that the microscope be used at its highest efficiency. Conventional microscopes equipped with glass (as opposed to quartz) optics are suitable for use in fluorescent antibody (FA) studies. Although achromatic objectives are acceptable, brighter images are obtained with apochromatic or fluorite lenses which possess higher numerical apertures (NA).

3-29. Dark-field condensers are often used for FA work. When working with microorganisms in otherwise clean smears, a dark-field condenser makes it possible to illuminate the field with white light in order to make visible certain organisms that may not be visible otherwise because of lack of fluorescence. With a bright-field condenser, white light is likely to be too intense for you to see unstained organisms. In general, you can get a darker background with a given level of fluorescence using the dark-field condenser.

3-30. The choice of a monocular or a binocular head for the microscope depends upon individual circumstances. A binocular head may yield images no more than 40 percent as bright as a monocular head. In some types of FA work this sacrifice of brightness is well worth the increased comfort of working with a binocular microscope; in other cases such loss of light could not be tolerated. You can use a standard microscope stand; however, the microscope should be equipped with a mechanical stage and a substage condenser mount that can be centered.

3-31. The most important consideration in choosing objectives, aside from general quality and workmanship, is numerical aperture (NA), since the higher the aperture the greater the light-gathering power. For example, the combination of a 20X ocular system and a 50X oil objective lens (NA = 1.00) provides brilliant bright—or dark—field images and gives 1000X magnification.

3-32. Very bright light sources are needed to produce visible fluorescence with the minute amounts of fluorescein involved in FA reactions. The suitability of a source, when high-power objectives are used, is determined primarily by its brightness per unit area rather than by the total luminous output. Therefore, small, highly concentrated sources may be preferable to large, more diffuse sources with much greater total luminosity. High-pressure mercury arcs inclosed in quartz envelopes are excellent light sources. They are available as small arcs with high intrinsic brightness which emit a significant percentage of their energy in that portion of the spectrum most suitable for fluorescein, i.e., the ultraviolet and the blue.

3-33. You must use filters in fluorescence work because the brightness of a fluorescent image is very low in comparison to the excitation light. To prevent masking of fluorescence emission, the excitation light must be removed before it reaches your eye. To do this, you place a primary filter between the lamp and the object so that only fluorescence-exciting wavelengths are passed, and you place a secondary filter between the object and your eye so that you see only wavelengths characteristic of the fluorescence. A properly matched pair of filters appears completely opaque when held in tandem against a strong light.

3-34. Three general types of lighting systems may be used for fluorescein: (a) near ultraviolet exciting light between 350-400 mu, secondary filter colorless but opaque to ultraviolet; (b) blue-violet exciting light between 400-450 mu, secondary filter distinctly yellow; (c) combined ultraviolet and blue-violet exciting light between 350-450 mu, secondary filter distinctly yellow.

3-35. Combination (a) is most useful in working with agents like viruses, whose specific staining may not be much more intense than the autofluorescence of the background, and with agents such as certain fungi which have strong autofluorescence. In these cases, the colorless secondary filter permits you to distinguish between blue gray or white autofluorescense and the specific yellow-green color of fluorescein. When working with agents that stain intensely, such as most bacteria and protozoa, brighter fluorescence is obtainable with combination (c) even though the yellow secondary filter limits observation to the green-through-red range. Combination (b) is useful because the same filters can be used for both bright and dark-field illumination.

3-36. Although serum has been labeled and used successfully for staining, this practice is usually less desirable than labeling a globulin fraction for two reasons. First, it is wasteful of labeling agent which attaches itself to nonantibody as well as to antibody protein. Second, it may increase nonspecific staining since serum is composed of several components. To isolate the desired fraction, you may follow standard procedures for separating globulin from other serum proteins. For routine uses of FA in the diagnostic laboratory there is no reason to use highly refined techniques for isolating narrow fractions of globulins. Precipitation with half-saturated ammonium sulfate is a simple and highly satisfactory procedure that you can perform in any laboratory without special equipment.

3-37. Most FA work has been based on the use of fluorescein isocyanate as the labeling agent. Use of this reagent involves certain practical difficulties because of its unstable nature in the presence of moisture. Recently, the isothiocyanate derivative of fluorescein was synthesized and described as a substitute for the original compound. The new reagent is available commercially as a stable, dry powder which can be kept on hand and used with a minimum of difficulty. Globulins can be labeled now in any laboratory, in contrast to the situation a few years ago when only well-equipped centers could undertake the process. Under ordinary circumstances fluorescein is probably the best label because of its high efficiency and because it fluoresces with a color to which the eye is most sensitive. In addition, its yellow-green color is rarely encountered as autofluorescence in normal tissue.

3-38. An estimate of the efficiency with which a globulin solution has been labeled is provided by determination of the fluorescein-protein (F/P) ratio. Measurements of dye and protein concentrations of a dialyzed conjugate allow the expression of a ratio which indicates the amount of dye attached to the protein. This can be expressed in terms of micrograms (μg) of fluorescein per milligram (mg) of protein. In general, using older methods of labeling with fluorescein isocyanate, F/P ratios of 4:5 are considered satisfactory.

3-39. The effect of fractionation and labeling procedures on the antibody titer of sera employed in FA work has not been studied intensely, but globulins conjugated with fluorescein rarely show more than 50 percent reduction of the original serum antibody titer. Labeled globulins seem to have the same storage characteristics as unlabeled globulins. Either labeled or unlabeled, they should be stored undiluted since protein denaturation is accelerated in dilute solution. As with sera in general, if preparations are to be kept for long periods, it may be well to lyophilize or freeze them. Lyophilized, labeled globulins usually reconstitute readily into clear solutions. Merthiolate can be added to globulin to give a final merthiolate concentration of 1:10,000 to inhibit bacterial growth, particularly if conjugates are to be kept at 5° C. for any length of time. Contaminants, particularly psychrophilic bacteria, may severely reduce the staining titer of a conjugate.

3-40. *Sorption of serum globulins.* Improving serologic specificity of FA reactions and reducing nonspecific staining reactions are problems similar in many respects to the problems encountered in other serological procedures. Generally, they are of more importance in FA work. Sorption can be performed in three ways—with tissue powders, with ion-exchange resins, and with heterologous, serologically related antigens. In most cases involving staining of antigens in tissue sections it has been found essential to sorb conjugates with tissue powders, e.g., hog liver powder, in order to reduce nonspecific staining. Sorption with tissue powders is not necessary, however, in staining bacteria in smears made from cultures or from tissues.

3-41. Ion-exchange resins for sorption of conjugates are particularly useful in removing the material responsible for nonspecific staining of leucocytes, which has been a widely encountered difficulty.

3-42. Using routine procedures, labeled or unlabeled antiglobulin or antiserum may be sorbed with packed cells of bacteria which cross-react with the antibody being tested. Antibody solutions prepared against viruses, protozoa, or fungi may be rendered more specific by sorption with appropriate antigens.

3-43. *Application.* FA staining is theoretically applicable to any system in which specific antigen-antibody reactivity occurs, regardless of whether the reaction is demonstrable by any other serological method. In practice, the matter is not so simple. No two pathogenic species have identical relationships to their respective hosts or antibodies. Thus, each

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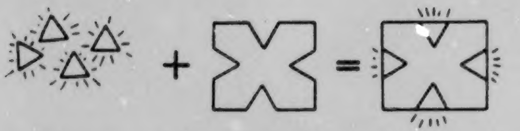

Figure 7. Schematic of direct staining with fluorescent antibody.

clinical or environmental specimen containing pathogenic organisms has its unique relationship to the FA that is used for detection. These differences commonly take the form of variations in the specificity, sensitivity, and rapidity with which the organisms may be identified. There is one basic difference between serological tests employing FA and conventional tests such as agglutination and precipitation. The former involves a single-stage antigen-antibody reaction, whereas the latter requires the formation of lattices resulting from secondary reactions that build aggregates to macroscopic size.

3-44. As with many other diagnostic tests, FA examinations are no better than the controls you set up for them. In establishing these controls, you must consider several variables. Regardless of the type of FA test employed, you have to keep in mind the possibility of encountering natural antibodies in the serum of normal animals. Natural antibodies for *Escherichia coli, Proteus, Corynebacterium diphtheriae,* and the staphylococci have been noted. Such reactions are important in the interpretation of FA tests, especially since these relationships are not always demonstrable by agglutination or other types of serologic tests.

3-45. *Direct technique.* Direct staining of antigens with FA solutions is the simplest form of the test. Apply labeled antibody to smears of antigen fixed on slides. After an interval of time previously determined for each system under study, wash away the excess antibody, mount the preparation, and examine it with a fluorescence microscope. In diagnostic testing, you use the direct test to identify unknown antigen by employing known labeled antibody as the staining agent. The principle of direct staining is shown in figure 7.

3-46. Direct staining of particulates by FA does not present serious difficulties in interpretation provided the labeled globulin has a good staining titer, does not give nonspecific staining, and shows a minimum of cross-staining reactions with heterologous antigens. You must get this information about the labeled globulin for each system before using the FA solution with unknown

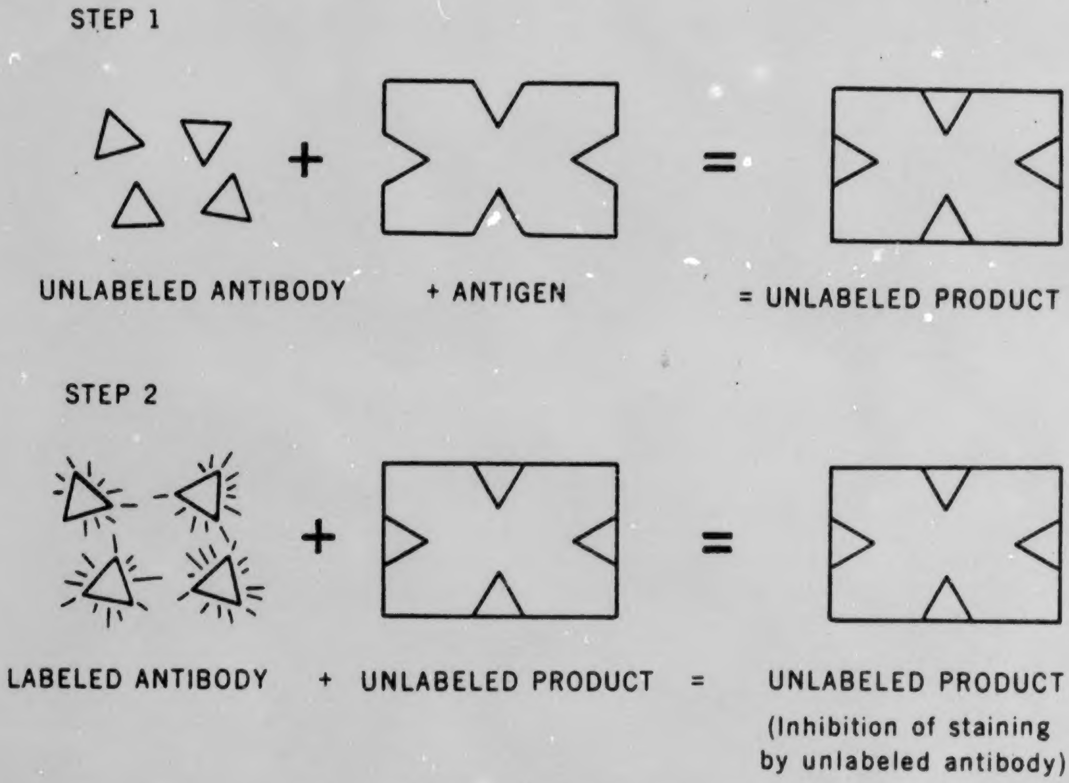

Figure 8. Schematic of fluorescent antibody inhibition staining reaction.

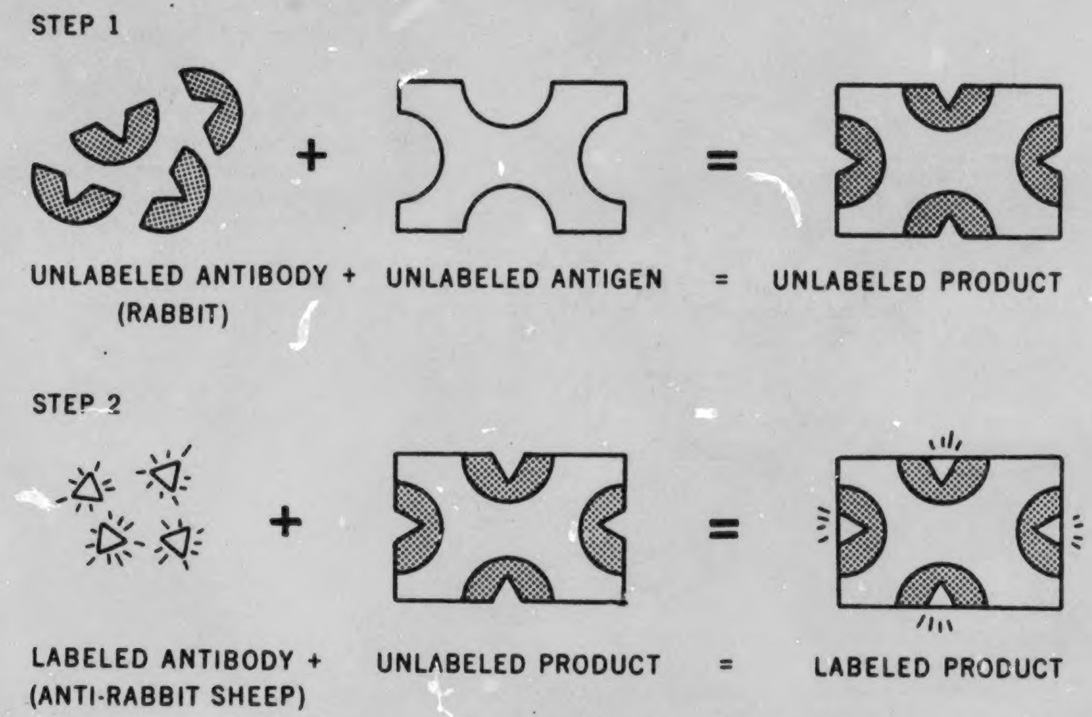

Figure 9. Schematic of indirect fluorescent antibody staining.

antigens, since the information is necessary to establish the degree of reliability of the technique.

3-47. *Inhibition.* This procedure is based on the phenomenon of blocking specific antigen-antibody reactions by first exposing the antigen to a different aliquot of homologous antibody solution. For example, if a smear of streptococci is exposed to specific unlabeled antibody, the bacteria become saturated with antibody. If the same smear is then exposed to specific labeled antibody, no reaction occurs and the organisms remain nonfluorescent. The principle of this procedure is illustrated in figure 8.

3-48. *Indirect method.* Indirect FA staining is a modified Coombs type of reaction in which the binding of unlabeled antibody to antigen is visualized by means of a "second stage" FA indicator rather than by "second stage" serologic reactions such as agglutination. It is helpful to remember that the unlabeled antibody plays a dual role—acting as antibody in the primary reaction and as antigen in the secondary reaction. The indirect fluorescent antibody staining principle is shown in figure 9.

3-49. Indirect FA tests allow you to determine either the identity of an unknown antigen or the antibody content of an unknown serum. In the first case, you react an unknown antigen with a known unlabeled antibody solution from a given animal species. Remove excess antibody and treat the preparation with labeled antiglobulin directed against globulin of the species used in the initial exposure. Fluorescence indicates a reaction between unknown antigen and antibody globulin of the primary reagent. Indirect determination of the identity of an antibody is the method used in the fluorescent treponemal antibody (FTA) test which will be described in Chapter 4.

3-50. Complement staining is similar to the indirect procedure except that the antiglobulin conjugate (secondary reagent) is not directed against the species supplying the antiserum (primary reagent) but rather against the species supplying complement. You add complement to the antiserum during the first stage of the reaction. As in the indirect method, complement staining permits identification of either an unknown antigen or an unknown serum. Complement staining is diagrammed in figure 10.

3-51. In complement staining, you apply inactivated primary reagent antiserum and a fixed amount of guinea pig complement to the antigen simultaneously. After the usual incubation and washing, you apply labeled secondary reagent (anti-guinea pig complement) and let the preparation incubate again. Perform the final washing and examination as described for the other methods. A disadvantage of the method is that there is greater difficulty in eliminating background nonspecific staining than in either the direct or the indirect

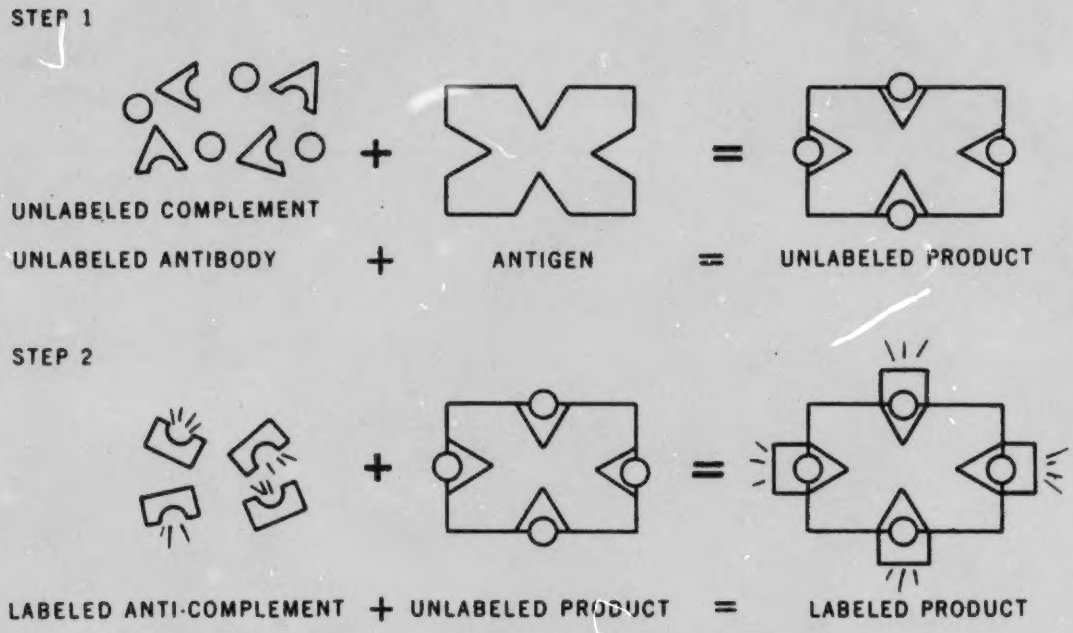

Figure 10. Schematic of complement staining with fluorescent antibody.

method. You can usually overcome this by pretesting and selecting individual guinea pig sera that do not give nonspecific staining. Additionally, before you can successfully screen unknown sera by complement staining, you have to determine that antibody in the serum of each species being tested will fix guinea pig complement when reacting with the antigen under study.

3-52. *Specificity.* After you have stained an antigen by the direct, indirect, or complement test, you have to prove that the observed staining is specific—that is, that the observed result represents a reaction between antigen and its specific antibody. You must prove specificity for each new conjugate prepared in the laboratory even when similar conjugates have been evaluated previously. The proofs given in the following checklist are essential in confirming the specificity of the reaction.

a. Uninfected tissue or uninoculated culture medium does not stain. Tissue or culture medium containing unrelated organisms does not stain.

b. If, in the system being studied, antigen concentration increases with the time of incubation, the degree of fluorescent staining increases correspondingly.

c. Normal sera (in the indirect or complement test) or conjugates of normal sera (in the direct test) do not stain the antigen.

d. Staining of antigen (in the direct test) is inhibited by pretreatment of the smear with unlabeled specific antibody or by dilution of the conjugate in specific unlabeled antibody.

e. Staining (direct, indirect, or complement test) is inhibited by dilution of the reagent (either serum or conjugate) in homologous antigen suspension or by sorption of the reagent with homologous antigen prior to staining.

CHAPTER 2

Agglutination Tests

WHEN WE WATCH blood cells agglutinate, we are observing a highly complicated phenomenon. If bacteria are agglutinated by mixing a drop of a broth culture with a specific antiserum, do you suppose the agglutination you observe is any simpler? Very unlikely. In fact the process is just as complicated as when blood cells clump.

1. How many intermediate steps do you suppose are involved in this process? As you can imagine, very many intricate and complex processes are occurring. In some instances the reactions are immediate and simultaneous, whereas in others certain reactions occur only after the completion of prior reactions. Also, consider special requirements, such as critical temperature, electrolytic media, and optimum concentration of the antigen and antibody.

2. In the serology laboratory wide use is made of the agglutination phenomenon. Many diagnostic tests are based on this process. This chapter discusses some of the tests in which the agglutination reaction aids in deciding whether or not the patient has been exposed to certain infectious diseases. We shall introduce you to these tests by presenting a few *classical procedures* that will help you understand the nature of the antigens and antibodies which give rise to agglutination. We shall also indicate precautions to be taken to insure properly controlled tests. Where possible, new methods and techniques will be discussed, their components analyzed, and their applicability assessed.

4. Serological Tests for Infectious Mononucleosis

4-1. Infectious mononucleosis (IM) is a disease frequently tested for in the serological laboratory. This disease has been investigated and reported upon frequently since its description as a clinical entity almost 50 years ago. Much of the interest in this disease over the years has been due to the search for a probable causative agent. Although it rarely kills and seldom cripples, it strikes down the young in great numbers, sometimes in almost epidemic proportions. Persons with infectious mononucleosis exhibit several varying symptoms depending on the severity of the infection and the body's response. Generally, this disease causes the patient to develop a fever and an increased white blood cell count with atypical lymphocytes. During the course of the disease the patient usually exhibits adenopathy (swelling of lymph nodes), splenomegaly (enlargement of the spleen) and occasionally splenohepatomegaly (both spleen and liver enlargement). Although these terms describe some of the symptoms that may beset the patient, they do not always occur. Some people have the disease in a mild form with few of the described symptoms but undergo a strong antibody response, as evidenced by high serological titers. Other persons may have the symptoms in their severest form with little or no antibody response. As you can see, situations such as these tend to be confusing. One writer went so far as to describe the disease as a kind of controlled leukemia.[1] This is certainly understandable when we examine a blood smear from a patient with the disease and see, typically, a wide assortment of definitely abnormal lymphocytes.

4-2. In discussing infectious mononucleosis we shall emphasize several aspects of interest to the serologist. First we will discuss heterophile and other related antibodies. Then we shall examine present thinking concerning the cause of this disease. We will also review the classical or heretofore standard tests for diagnosing the disease. Finally we will summarize the newer slide tests for IM.

4-3. **Heterophile Antibodies.** We may divide antibodies into two broad categories based on the source of the antigen that caused their production. If an antibody reacts with antigen from *all* members of a certain species of animal such as all sheep, all humans, or all horses, but reacts *only* with antigen from that certain species, then it is an *isophile antibody.* Antibodies that react with antigens from several different species of organisms are *heterophile antibodies.* The word heterophile is derived from the Greek words *eteros*, meaning other, and *philein*, meaning to love. They may be thought of as antibodies which will react with antigens other than the specific antigen that caused them to be produced. Antigens capable of stimulating the production of heterophile antibodies are called heterophile antigens.

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[1] William Dameshek and F. Gunz, *Leukemia* (New York and London: Grune and Stratton, 1964), p. 566.

4-4. Isophile antibodies do not occur naturally. They can be produced by injecting suitable antigens into an animal. If a rabbit is given an injection of sheep cells, it will develop several different kinds of antibodies. Some of these will be specific for cell wall nuclear remnants and other cell proteins. A small number of these antibodies will be exclusively specific for sheep cells. These anti-sheep cell antibodies are isophile antibodies, and as such will react with all sheep cells. Some of the other antibodies produced by the rabbit will be heterophile antibodies. These antibodies will react with antigens from several sources.

4-5. Heterophile antigens are present in a variety of animals, plants, and bacteria. The plant and bacterial heterophile antigens get into the body through ingestion, inhalation, or infection in which they are a component of microorganisms. An immune response to these antigens produces the naturally occurring heterophile antibodies called Forssman antibodies. On the other hand, another type of heterophile antibody is sometimes produced as a result of receiving an injection of horse serum such as in antitoxin treatment of diphtheria. Antibodies produced in this fashion are called serum sickness antibodies. Infectious mononucleosis stimulates the production of yet another heterophile antibody.

4-6. Just as the ABO system is only one of the human blood groups, several types of heterophile antigens exist. Heterophile antibodies are similar in that they all react with common antigens in certain conditions, but they also differ very much. In some of these systems the antibodies are hemolysins and hemolyze heterophile antigen-carrying cells. In others, the antibodies are hemagglutinins and agglutinate the heterophile antigen carrying cell. Sometimes, it is a bacterial agglutinin or a precipitin which reacts with certain lipopolysaccharides.

4-7. The three best known heterophile systems are those of Forssman, serum sickness, and infectious mononucleosis. Forssman discovered in 1911 that rabbits injected with emulsions of guinea pig kidney, adrenals, liver, testes, and brain produced antibodies that hemolyzed sheep cells in the presence of complement and agglutinated sheep cells in the absence of complement. This antigen became known as the Forssman antigen and the antibody it produced as the Forssman antibody. Guinea pig red blood cells and serum did not contain the antigen. Further work showed that the Forssman antigen occurred, inherently, in a variety of organisms. It is found in human Group A and B cells and sheep erythrocytes. It is also found in the horse, dog, cat, mouse, fowl, tortoise, and certain other animals. It is absent in both red blood cells and organs of rabbits, cows, pigs, and rats. Microorganisms possessing the antigen include certain strains of *Salmonella, Pasteurella, Shigella, Diplococcus,* and *Bacillus.*

4-8. Serum sickness antibodies are produced when humans are given an injection containing horse serum. These antibodies react with sheep cells to produce hemagglutination. Since the horse is a source of Forssman antigen you could easily assume that the antibodies in serum sickness were of the Forssman type. This is not so. Serum sickness antibodies differ from Forssman antibodies in several qualities, as you shall see when we discuss serological tests for heterophile antibodies. An injection of horse serum produces antibodies in very much higher titer than is normally found with Forssman antibodies. This property, plus other differences of serum sickness antibodies, indicates that horse serum contains more than one type of heterophile antigen. A patient with serum sickness might therefore possess Forssman antibodies in relatively low titer and serum sickness antibodies of a relatively higher titer.

4-9. The infectious mononucleosis type of antibody is also classed as heterophile because it reacts with certain heterophile antigens. We can detect this antibody using the traditional sheep cell hemagglutination test. An anti-sheep agglutinin as well as an anti-sheep hemolysin is present in the serum of most patients having infectious mononucleosis. The hemolysin is not observed in the sheep cell hemagglutination test because the hemolysin requires complement to react. Inactivation of complement in the test specimen keeps the test from showing a hemolytic reaction.

4-10. Studies by A. A. MacKinney, as reported in 1968, have shown fairly conclusively that atypical lymphocytes are actively involved in the synthesis of immunologic macroglobulin. His observations first showed that the atypical lymphocytes were undergoing cell division at a much more rapid rate than normal peripheral leukocytes. Using radioactive isotopes and column separations, he was able to measure the amount of macroglobulin synthesis products present in these cells. The specific type of globulin was identified by the precipitin reaction of the immunodiffusion technique. The predominating immunoglobulin was observed to be IgM. The significance of this study is that it suggests that atypical lymphocytes are able to synthesize heterophile antibody.

4-11. **Etiology of Infectious Mononucleosis.** Researchers have searched diligently for the agent or agents causing infectious mononucleosis. Many possibilities have been considered. Most have been discarded as untenable. Bacterial agents were easily eliminated. No unusual increase in bacterial organisms is noted even in the most severe forms of this disease. A virus of some kind was considered the most likely cause by most workers. The fact that most viral infections produce adenopathy and atypical lymphocytes correlates very well with conditions found in infectious mononucleosis. Several microorganisms, including *Listeria monocytogenes*, have been isolated from some cases of IM, but not all cases. The observation that lymphocytes produce an IgM immunoprotein is further implication of a virus as the agent.

4-12. The work by Epstein and Barr of London in 1964 led to findings of major significance in pinning down the virus responsible for IM. Their work with tissue cultures from cell lines of Burkitt's East African malignant lymphoma led to the discovery of a herpes-like virus within successive generations of lympho-blast cells. The lymphoma cell line was designated Epstein-Barr (EB) and the herpes-like virus was designated Epstein-Barr virus (EBV). Further studies by other workers concerning the serological specificity of the EB virus have shown that it, or a closely related one, is widespread throughout the world.

4-13. A few years ago Drs. Henle and Diehl at the University of Pennsylvania studied detection of the EB virus, its relationship to other diseases, and its behavior in different groups of people. Quite randomly they tested the serum of one of their technicians who was recovering from an infectious mononucleosis infection. The convalescent serum specimen showed antibody to EBV, as opposed to a negative specimen taken prior to her illness. With these facts in hand other acute and convalescent specimens were taken from patients with IM. The antibody regularly appeared and remained high in titer for a long period. They were also able to identify the viruses within lymphocytes of afflicted persons. Further collaboration with Dr. Niederman confirmed their findings. Dr. Niederman had a stock of frozen paired sera from college students who reported to Yale University negative for IM by traditional tests, and who subsequently developed the disease. He, consequently, had a good stock of before and after specimens. Studies performed on these specimens showed a definite development of antibodies to EBV. One later study on a well-documented case showed that antibody was still present in a patient who had the disease 37 years before testing.

4-14. Studies by A. S. Evans have shown that IM infections in early childhood may not be recognized and heterophile antibodies may not develop. Most teenagers and young adults who get the disease usually develop the ordinarily associated symptoms and appearance of antibody. Babies and very small children frequently show no immune response. Theories have been advanced that the EBV and similar viruses are common within the body. Unusual circumstances or conditions cause them to become disease agents. This may well be the case. More work is now going on to further define the EBV and its relationship to IM. At this writing, the EBV or a close relative seems to be the most likely agent causing infectious mononucleosis.

4-15. **Paul-Bunnell Test.** The classical sheep cell test for heterophile antibodies was developed from studies begun in 1929 by Davidsohn and in 1932 by Paul and Bunnell. Dr. Davidsohn's study showed that serum sickness produced anti-sheep cell antibodies in high titer. He further found that these antibodies could be partially absorbed by exposing them to guinea pig kidney and completely absorbed by beef erythrocytes. Paul and Bunnell found that the heterophile antibodies of infectious mononucleosis were not removed by guinea pig kidney but were completely absorbed by beef erythrocytes. They also observed that infectious mononucleosis caused the production of antibodies in higher titer than either those of Forssman or those caused by serum sickness. This led them to devise a presumptive test for infectious mononucleosis using a 2-percent suspension of sheep cells—a source of antigen.

4-16. In the presumptive test you first heat a serum specimen to remove complement; if complement is not removed, it lets sheep hemolysin react and causes hemolysis that might give misleading test results. After inactivation of complement (56° C. for 30 min.), serially dilute the serum specimen and add a 2-percent suspension of fresh sheep cells in saline. The sheep cells act both as a source of heterophile antigen and as an indicator of reaction. Allow the tubes to stand at room temperature (20° C.) for 2 hours. Read the tubes for visible agglutination; the titer of antibody present is the reciprocal of the last tube showing agglutination. A titer of 56 in the absence of other findings such as atypical lymphocytes and clinical symptoms is insignificant and for all practical purposes the specimen is considered negative for IM antibodies. If other findings are present and a titer of 224 or higher is found, the presumptive test is considered positive.

4-17. Highest titers are usually found during the second to fourth week of infection. Some people, severely ill with IM, have low or moderate titers; while others, only slightly affected, have very high titers. In some instances persons with a negative or low titer specimen still show both atypical lymphocytes and the usual clinical signs of the infection. Infectious mononucleosis produces anti-sheep cell antibodies in only 50 to 80 percent of

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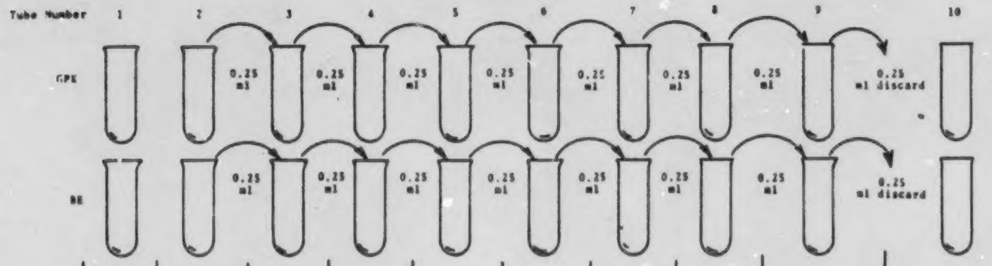
Table 4
Davidsohn differential heterophile test



Tube Number	1	2	3	4	5	6	7	8	9	10
Saline ml	None	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Supernatant Fluid (1:5 dilution of serum)	0.25	0.25	---	---	---	---	---	---	---	---
Serum Transfer ml	---	---	0.25 milliliters of							None (Control)
			1:10	1:20	1:40	1:80	1:160	1:320	1:640	
Dilution After Serum Transfer	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	---
Sheep Corpuscles ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total Volume ml	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Final Dilution of Serum	1:7	1:14	1:28	1:56	1:112	1:224	1:448	1:896	1:1792	---

Mix thoroughly and keep at room temperature for 2 hours; make readings.

those persons infected. Very young children may have very little antibody response. Therefore, in cases exhibiting typical signs but showing a titer of less than 224, a differential heterophile test is required. For example, a titer of 28 or 56 could be due to natural Forssman, serum sickness, or IM antibodies.

4-18. **Davidsohn Differential Test.** A test to differentiate the three common types of heterophile antibodies was devised by Dr. Davidsohn as a continuation of his work with the serum sickness antibodies that he reported upon in 1929. As mentioned before, he found that serum sickness antibodies were partially absorbed by guinea pig kidney (GPK) and completely removed by beef erythrocytes (BE). In the differential test, you

Table 5
Absorption patterns of heterophile antibodies

Type Antibody	GPK	BE
Serum Sickness	Absorbed	Absorbed
Forssman	Absorbed	Not Absorbed or partial
Infectious Mononucleosis	Not Absorbed or slightly	Absorbed

Table 6
Differential test interpretation

Presumptive test titer	Differential test after adsorption with ----		Interpretation with regard to infectious mononucleosis
	Guinea pig Kidney	Beef red cells	
1:224	1:112	1:7	POSITIVE
1:224	1:56	0	POSITIVE
1:224	1:28	0	POSITIVE
1:224	1:14	1:112	NEGATIVE
1:224	1:7	1:112	NEGATIVE
1:56	1:56	0	POSITIVE
1:56	1:28	0	POSITIVE
1:56	1:14	0	POSITIVE
1:56	1:7	0	POSITIVE
1:28	1:28	0	POSITIVE
1:28	1:14	0	POSITIVE

inactivate the patient's serum complement as before. Then mix an aliquot of test serum with a suspension of macerated guinea pig kidney, and mix another aliquot with a suspension of boiled beef erythrocytes. Allow both mixtures to stand at room temperature for 3 to 6 minutes, then spin the mixtures at 1500 r.p.m. for 10 minutes or until the supernatant is clear. Remove the supernatant from each to a separate clean test tube. Only the IM antibodies are left unabsorbed in the supernatant solution. Remember that in the process of absorbing the unwanted Forssman and serum sickness antibodies with GPK and beef erythrocytes, the original serum specimen becomes diluted.

4-19. Test aliquots of the supernatant solutions against sheep cells for agglutination in a modified form of the presumptive test shown in table 4. Table 5 shows the absorption pattern you can expect. Results are usually reported as a titer of each type of absorption, such as GPK 1:112 BE 1:7. In positive tests the GPK supernatant solution differs by no more than a three-tube drop in titer as compared to the presumptive test. The BE supernatant solution will give a very low or negative result in tests positive for IM. Table 6 gives a random sampling of a few possible combinations of GPK and BE test results. Other combinations are possible. For instance, the presumptive test may be 1:56 with a

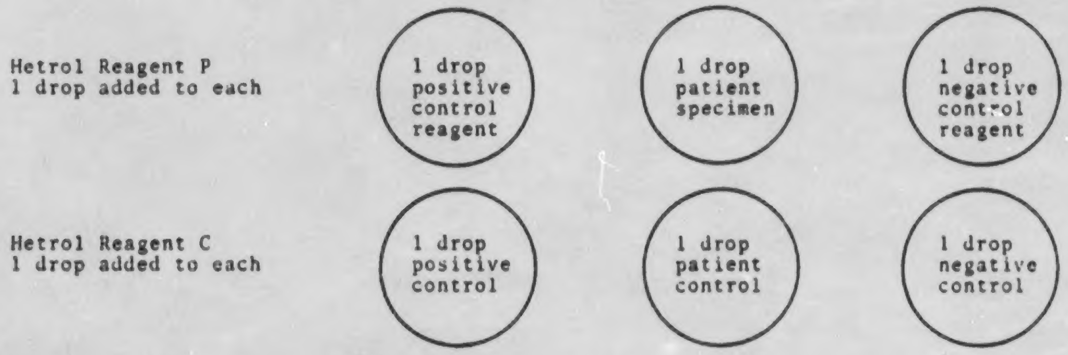

Hetrol Reagent P
1 drop added to each
1 drop positive control reagent
1 drop patient specimen
1 drop negative control reagent
Hetrol Reagent C
1 drop added to each
1 drop positive control
1 drop patient control
1 drop negative control

Figure 11. Hetrol test schematic.

Table 7
Hetrol test reactions

	Hetrol Reagent P	Hetrol Reagent C
Negative Serum	No Clumping	No Clumping
Negative Control	No Clumping	No Clumping
IM Antibodies	Clumping	No Clumping
Positive Control	Clumping	No Clumping
Other Heterophile Antibodies	Clumping	Clumping

hand, you will rarely encounter presumptive tests of 1:224 or higher that yield a negative IM differential test pattern. Misleading test results may be due to incomplete inactivation of complement in the specimen.

4-20. **Other Tests for IM.** Various new tests have been devised to test for the presence of heterophile antibodies. These tests are generally one-step or two-step rapid slide tests. Before discussing some of the more popular ones, we should point out that serological tests are invalid without proper controls. Just as tube tests always include controls, so also should slide test procedures. These controls are essential to proper interpretation of the test reaction. This is especially true of slide tests because of the shortened reaction time of slide test procedures. Controls are also necessary because of increased concentrations of cells and solutions. Thick mixtures and heavy suspensions used in slide test procedures can be difficult to read correctly. Controls help in this respect. Slide tests, as a rule, are not as sensitive as tube tests that incorporate longer incubation and testing times. However, if performed properly, especially following the manufacturer's direction, the slide methods usually give results within acceptable limits of error.

4-21. *Mono-Test* ®.[2] This test is a 2-minute slide test using standardized and specially treated sheep cells. The patient's serum may be used directly or with complement inactivated. The specimen should be as fresh as possible. If it must be kept more than 24 hours before testing, store it frozen. Perform the test by mixing one drop of the patient's serum with one drop of Mono-Test Reagent. Positive and negative control specimens are set up and tested along with the test specimen. Use a clean applicator to mix each specimen. Rotate the slide and read it for agglutination within 2 minutes under indirect light or against a dark background. Positive test results are indicated by coarse agglutination.

4-22. *BactoHetrol Slide Test* ®.[3] This test is supplied as a kit composed of the following reagents:

- Hetrol Reagent P—a stabilized suspension of erythrocytes used in screening sera as a presumptive test for IM.
- Hetrol Reagent C—a stabilized erythrocyte suspension used to confirm a positive heterophile test with Reagent P.
- Hetrol Positive Control—a reagent that gives a

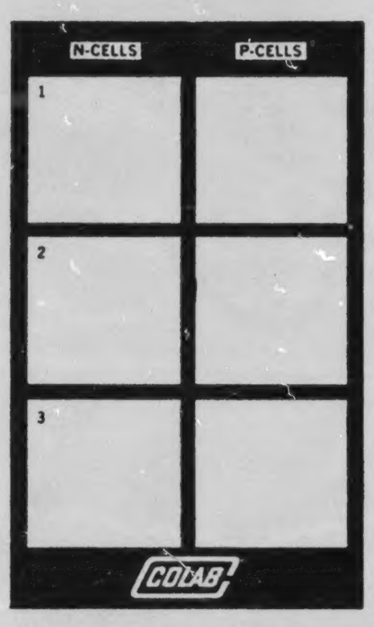


Figure 12. Mono-Stat test slide.

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[2] Registered Trademark—Wampole Laboratories, Stamford, Conn.

[3] Registered Trademark—Difco Laboratories, Detroit, Mich.

Table 8
Mono-Stat test interpretation

	N-cells (Native)	P-cells (Papain)
Reagent Control	No Clumping	No Clumping
Normal Serum -- Titer less than 1:64	No Clumping	No Clumping
IM Antibodies	Clumping	No Clumping, or weak and delayed clumping
Other Heterophile Antibodies	Clumping	Rapid Clumping

positive test with Reagent P and a negative test with Reagent C.

- Hetrol Negative Control—this reagent gives a negative test with both Reagent P and C.

4-23. Perform the test by mixing reagents and serum as indicated in figure 11. A special agglutinations slide is supplied with each kit. Mix the drops in their appropriate squares with an applicator and rotate the slide for 2 minutes. As you can see in table 7, sera positive for IM antibodies can be differentiated from IM negative sera.

4-24. *Mono-Stat Test* ®.[4] This test comes from the manufacturer as a kit composed of a premarked agglutination slide; a reagent control (negative control), a vial of native or untreated stabilized sheep RBCs (N-cells) and a vial of papainized sheep RBCs (P-cells). The principle of this test is that papain inactivates the IM antibody receptor cites on the papainized cells. This causes a delayed reaction by IM antibodies and the papainized cells (P-cells). IM antibodies react rapidly with the N-cells. Other types of heterophile antibodies react rapidly with the P-cells and a little slower with the N-cells. The manufacturer states that this test can be performed on unactivated serum and that oxalated, E.D.T.A., heparinized, or citrated plasma may be used.

4-25. Perform the test by adding N-cells and P-cells to appropriate squares on the agglutination slide illustrated in figure 12. Add reagent control to row 1, and patient serum or plasma to either row 2 or 3. Reactions are interpreted as shown in table 8. If you observe clumping in row 1 (reagent control) the test is invalid and indicates that the test cells are not reacting properly.

4-26. *Monosticon* ®.[5] This test is a modified form of Davidsohn's differential test and is supplied as a kit containing the following reagents: (1) Beef and sheep antigen suspension, (2) guinea pig and sheep antigen suspension, (3) sheep antigen, and (4) positive control. A special agglutination slide is supplied. Interpret the test as illustrated in table 9. A quantitative test kit is also supplied and positive specimens may be titered in terms identical to the standard differential test.

Table 9
Monosticon test interpretation

Reagent	IM	Negative	Forssman Antibody
Beef and sheep	No Clumping	No Clumping	Clumping
Guinea pig and sheep	Clumping	No Clumping	No Clumping
Sheep	Clumping	No Clumping	Clumping
Positive Control and sheep	Clumping	Clumping	Clumping

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[4] Registered Trademark—Colab Laboratories, Chicago, Ill.

[5] Registered Trademark—Oragon Inc., West Orange, N.J.

4-27. *MonoSpot Test* ®.[*] This test is supplied in kit form and is basically composed of two reagents and a vial of preserved horse cells. A special agglutination slide is also included. The reagents are a specially prepared guinea pig kidney antigen (Reagent I) and specially prepared beef erythrocyte stroma antigen (Reagent II). Mix the patient's serum with each of the reagents. If IM antibodies are present they will be removed by Reagent II but not by Reagent I. An indicator composed of preserved horse cell is added to each mixture and a stronger reaction in Reagent I than in Reagent II indicates a positive test for IM antibodies.

4-28. *Horse erythrocyte tests.* Many of the new screening tests for IM antibodies are based on the increased affinity that IM antibodies have for the horse erythrocyte. In two recent papers the reactions of IM antibodies with horse cell and sheep cell indicator systems were reported.[7] These studies suggest that horse erythrocytes are a better indicator than sheep RBC in the differential test for IM. The spot test using horse erythrocytes, after absorption with guinea pig kidney and beef erythrocyte stroma, is described in detail in these studies. Titers in the horse cell indicator system are higher than those using sheep RBC. Also, horse cells have yielded significant titers while duplicate tests using sheep RBC gave negative or insignificant results.

5. Febrile Agglutination Tests

5-1. Prior to the development of modern bacteriological techniques, the cultivation of many of the common bacteria was difficult and haphazard. Such an organism as *Salmonella typhi*, which is routinely cultured now, was rarely isolated in active infections. Carriers of this organism were even more difficult to identify because the organism could not be cultured easily. Typhoid fever, the disease caused by *S. typhi*, was at one time the cause of great epidemics. Large segments of entire communities were at times affected by the disease.

5-2. Typhoid fever produces characteristically a very high fever. The disease is classified, along with several other fever-producing illnesses such as brucellosis, tularemia, and the various rickettsial infections, into a category known as the *febrile diseases*. Since culture techniques for identifying fever-producing microorganisms were not very practical, alternate methods of testing for presence of infection were developed by Weil, Felix, Widal, and others. These methods tested for the presence of antibodies against the various microorganisms causing a particular infection. These serological methods are referred to as *febrile agglutination* tests. In this section, we will discuss these tests with particular emphasis on development of the "classical test," the antigens and antibodies involved, and specificity of the reaction.

5-3. **Bacterial Agglutinins.** Run these tests on acute and convalescent specimens. In some febrile illnesses the antibody response does not appear until after the first week. Ten days to two weeks or more may be required before a significant response can be detected. Tests on samples collected at staggered intervals provide more meaningful test results. In some instances patients who have had febrile diseases such as typhus or brucellosis show an anamnestic reaction (incidental stimulation of a prior immunization) when tested for typhoid. In an anamnestic reaction the presence of typhoid organisms restimulates the body to produce antibodies against previous typhus or brucellosis infections. The presence of these antibodies tends to confuse the interpretation of the test unless you consider the patient's history and know some of the characteristics of the causative agents.

5-4. *Salmonella* organisms cause a group of febrile illnesses important because of their high morbidity and mortality. These diseases are very prevalent where sanitary standards are low. The diseases occur frequently in Central Europe, the Middle East, and Asia. They still occur occasionally in rural areas of the United States and Canada. Typhoid fever is caused by *Salmonella typhi*. Paratyphoid fever is caused by several strains including *S. paratyphi* A, *S. paratyphi* B, and *S. choleraesuis*. These diseases are routinely tested for in most laboratories.

5-5. The original Widal test, using live and motile *S. typhi* as antigen source, was devised to detect the antibodies of typhoid fever. The test was designed to be performed on a glass slide or any other handy clean surface, such as aluminum foil or nonabsorbent paper. This makes the test very adaptable to field conditions, especially since the specimen (a drop of whole blood) is allowed to dry. Prepare the specimen for use by adding a drop of saline and mixing until you get a faint orange tint. Mix a loopful of this mixture with a loopful of a 24-hour broth culture of live motile typhoid organisms on a glass slide. Seal the slide with a petrolatum-ringed coverglass and allow it to sit at room temperature for 30 to 60 minutes. As the reaction takes place, the first observable sign is the cessation of motility of the live typhoid organisms and finally the formation of aggregates. This original test procedure does not allow testing at several dilutions, which gives an idea of the amount of antibody response; nor does it allow for prozone reactions. False negative reactions occur occasionally. This technique has been modified many times. A variety of test procedures based on the original Widal reaction are available in most standard serology tests.

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[*] Registered Trademark—Ortho Diagnostics, Raritan, N.J.

[7] C. L. Lee and I. Davidson, "Horse Agglutinins in Infectious Mononucleosis," *American Journal of Clinical Pathology*, Volume 49, page 3, January 1968, and C. L. Lee, I. Davidson, and Panczyszyn, "Horse Agglutinins in Infectious Mononucleosis," *American Journal of Clinical Pathology*, Volume 49, page 12, January 1968.

5-6. The Widal procedure shows a very important fact about serology. This fact is that antigen-antibody reactions take place only in electrolytic solutions. The saline used to reconstitute the speciment is vital to the reaction. The number of bacteria in the antigen suspension also plays a significant part in the reaction. In most commercially prepared bacterial antigens, the bacteria are killed, usually with heat or phenol. The number of bacteria required to give a good reaction at a 1:80 dilution (the approximate dilution achieved in the original Widal test) varies with the strain of organism. Sometimes the number of organisms in a suspension is adjusted photometrically. This allows very precise antigen preparation for optimum antigen-antibody reaction.

5-7. Bacterial agglutination tests for the fevers caused by *Salmonella* organisms usually determine the presence or absence of antibodies against the following antigens: (1) *S. typhi* O and H, (2) *S. paratyphi* A, and (3) *S. paratyphi* B. Other *Salmonella* organisms can be tested for if desired. The decision to use other antigens depends on the prevalence of a particular group or strain within the testing area.

5-8. The O antigen is a somatic (cell body) antigen. It is protein in nature, is heat-resistant, and can be heated at boiling temperatures for more than 1 hour without damage. Agglutination produced when O antigen and antibody react is hard, granular, and sometimes difficult to break up even with vigorous shaking. The H antigen is a flagellar antigen derived from components in or on the flagella of the organisms. Flagellar agglutination produces a fluffy, cottonlike aggregate that is easily dispersed on shaking. The reaction results in loose intertwining of the flagella into soft knots in the presence of anti-H antibody.

5-9. The reason we test for two different antibodies (anti-O and anti-H) in typhoid fever is that this strain of *Salmonella* produces O antigens that are very highly group specific, and antibodies produced as a result of *Salmonella typhi* infection will react with all Group D *Salmonella* and occasionally with a few members of other groups. The antibodies against the H antigen are also group specific and, when present, confirm the fact that an *S. typhi* infection has occurred. Interpretation of these reactions is as follows:

- Reaction with O and H antigens—typical typhoid reaction.
- No reaction with O and H antigens—disease not typhoid or a Group D *Salmonella*.
- Reaction with O but H is negative—probably not typhoid or a very early infection.
- No reaction with O but H positive—nonactive disease or due to vaccination.

The anti-O antibody appears first in an infection, usually in 7 to 10 days. It disappears from the serum first. Anti-H antibodies appear later and remain in the serum longer, often for several years. A high titer for the H antigen and no reaction against the O antigen are to be expected if the patient has been immunized.

5-10. Antibodies against other *Salmonella* organisms, such as *S Paratyphi* A, B, and C, are detected similarly and their significance is generally the same as for typhoid. The paratyphoid fevers are usually less severe and not as lethal as typhoid fever. The usual test for them involves testing for antibodies against O antigen; however, flagellar antigens are available and make the test more definitive. Even gastrointestinal infections caused by *Salmonella* organisms can be detected, but usually by the time the antibodies have been formed and are in the serum in a detectable level, the patient is well and released from the hospital.

5-11. **Tests for Rickettsial Agglutinins.** In 1916 Weil and Felix discovered in the urine of a typhus fever patient a nonmotile strain of *Proteus* that was agglutinated by the patient's own serum They tested

Table 10
Rickettsial diseases

ORGANISM	DISEASE	CARRIER	REACTION		
			OX19	OX2	OX-K
R. prowazeki	TYPHUS Epidemic Brill's Disease	BODY LOUSE	[illegible] [illegible]	[illegible] [illegible]	[illegible] [illegible]
R. mooseri	ENDEMIC TYPHUS	FLEA	[illegible]	[illegible]	[illegible]
R. tsutsugamushi	SCRUB TYPHUS	MITES	[illegible]	[illegible]	[illegible]
R. rickettsi	SPOTTED FEVER GROUP ROCKY MOUNTAIN SPOTTED FEVER	TICK	Variable		[illegible]
R. conori	BOUTONNEUSE FEVER		"		"
R. australis	QUEENSLANDS TICK TYPHUS		"		"
R. sibiricus	NORTH ASIAN RICKETTSIOSIS		"		"
R. akari	RICKETTSIALPOX	MITE	[illegible]	[illegible]	[illegible]
C. burneti	Q FEVER	TICKS (airborne fecal material)	[illegible]	[illegible]	[illegible]
R. quintana	TRENCH FEVER	BODY LOUSE	[illegible]	[illegible]	[illegible]

serum from other typhus patients and observed that these sera also caused this strain of *Proteus* to agglutinate. Serum from uninfected persons did not cause agglutination. These tests showed quite clearly that there was an immune substance in the ill patient's serum that caused the bacteria to agglutinate. The *Proteus* bacillus was later found *not* to be the cause of typhus fever; it simply cross-reacted with rickettsial antibodies. The strain of *Proteus* bacillus was designated OX19. The O means the antigen is a somatic antigen of this nonmotile strain. The X19 refers to the particular strain number.

5-12. Two other strains of the *Proteus* bacillus give positive agglutination tests with other rickettsial organisms. In some cases, sera from several different kinds of rickettsial infections will cause the agglutination of the same strain of *Proteus*. The other two types of *Proteus* that react with sera from rickettsial infections are labeled OX2 and OX-K. Table 10 shows the reactions of *Proteus* antigens in rickettsial infections.

5-13. Some serums will contain predeveloped, low-titer antibodies against one or several of the *Proteus* strains. The titer of these antibodies should not increase significantly in non-cross-reacting rickettsial infections. You can eliminate these preformed antibodies as a source of error by taking specimens for testing several times over a period of several days. A significant rise in titer in this test is more diagnostic than a single specimen with high titer. Be extremely careful in handling specimens from suspected rickettsial infections, especially in a laboratory that attempts to culture the organisms. You can contract a fever quite easily through mishandling of laboratory specimens and refuse.

5-14. **Tube Versus Slide Tests.** As mentioned before, you can perform any of the febrile agglutination tests more accurately in a test tube than on a slide. The difficulty in doing tube tests is the large amount of time involved in diluting and pipetting the specimen and the long incubation time. You can cut diluting and pipetting to a minimum by preparing the original dilutions in large lots and distributing this to other tubes set up for specific tests. For example, in testing for *Salmonella* you can eliminate having to make one dilution for the O antigen test and another for the H antigen test. You can also save time by using an automatic pipetting apparatus to dispense the antigen suspension.

5-15. The original slide procedures utilized a suspension of the organism as antigen and were not very reactive nor accurate. Many antigen suspensions today consist of antigen adsorbed onto latex particles. These suspensions are very reactive and yield tests quite comparable to tube tests. They are particularly recommended as screening procedures. Several manufacturers recommend their antigens for slide quantitative tests in which the specimen is serially diluted and the tests are performed at approximately the same dilutions as in tube tests.

6. Other Agglutination Tests

6-1. Cold agglutination tests and *Streptococcus* MG tests are other agglutination tests performed in most serology laboratories. These tests are diagnostic for specific antibodies found consistently in primary atypical pneumonia (PAP). The cold antibody test is a hemagglutination test, and the *Streptococcus* MG test is a bacterial agglutination test. In both cases we identify antibodies in the patient's serum. In this section we shall discuss these tests and those conditions in which they are significant.

6-2. **Cold Agglutination Tests.** The cold agglutinin (or cold antibody) is present in a great many illnesses. It has been detected in primary atypical pneumonia, pregnancy, *Staphlococcus* bacteremia, tonsillitis, cirrhosis of the liver, influenza, and many other conditions. Cold agglutinins also occur naturally in low titer in most normal serum specimens. The disease with which we most often associate cold agglutinins is primary atypical pneumonia. In this illness, the antibodies appear during the first week after onset and reach their peak after 2 or 3 weeks. The antibody titer begins to fall after 30 days or so.

6-3. In the test for cold antibodies, allow the patient's blood to clot and remove the serum while the specimen is incubated at 37° C. This is necessary because antibodies of this type will agglutinate the patient's own cells if the temperature is low enough. The temperature range in which antibody attaches itself to its antigen is called the *thermal amplitude*. Cold antibodies have a thermal amplitude of 0° C. up to 37° C. There is some variation in the temperature at which cold antibodies from different people react. Cold antibodies from one individual may react only below 20° C., whereas those from another individual might react at temperatures up to 35° C. In the cold agglutination test, the testing temperature has been standardized at 2° to 4° C. because the majority of cold antibodies react best within this range.

6-4. Human group O cells are the source of antigen. Wash the cells and resuspend them as a 1-percent suspension. Serially dilute the patient's serum with saline. The dilution progresses 1:4, 1:16, etc. After setting up the test, place it in a refrigerator at 2° to 4° C. overnight. Immediate reading of test results after removing the tubes from the refrigerator is necessary, since warming may cause agglutination to disappear. Titers of 1:16 or less are considered nonsignificant. Titers of 1:32 or 1:64, if accompanied by a typical clinical picture, are significant. Higher titers on a single convalescent specimen are of course more diagnostic. The test is much more relevant if both acute and convalescent specimens are tested. A fourfold increase in titer is indicative of primary atypical pneumonia. Titers tend to vary with the severity of the disease; the more severe the disease, the higher the titer. However, for some unexplained reason some cases of severe disease show no elevation of cold antibody titer during the course of the illness.

6-5. It is generally accepted that *Mycoplasma pneumoniae*, a pleuropneumonia group organism, causes the primary atypical pneumonia associated with elevated cold agglutinins. Other organisms, such as viruses, fungi, and *Rickettsia burneti* are also known to cause nonbacterial pneumonia; however, these organisms have not been consistently associated with elevated cold agglutinins. *M. pneumoniae*, when first isolated by Eaton, Maiklejon, and Vanteric in 1944, was then labeled "Eaton Agent." Chanock, Mufson, and others in a study done in 1961 showed that it was present in 68 percent of their patients with primary atypical pneumonia. At one time *M. pneumoniae* was thought to be a streptococcal organism because it develops an L-form type of colony and because it reacts antigenically with antiserum of *Streptococcus* MG.

6-6. **Streptococcus MG Test.** Antigens of *Streptococcus* MG can be used to detect antibodies developed in a primary atypical pneumonia infection. In the test, a suspension of *Streptococcus* MG is reacted with the patient's serum and they are agglutinated if cross-reacting antibodies are present. The test is somewhat nonspecific in that cold antibodies produced in any condition will give a positive test.

6-7. The fluorescent antibody test (FA) is much more sensitive than an agglutination type of tube test and is the recommended method for demonstrating anti-*Streptococcus* MG antibodies. This technique, which was described in the previous chapter, can also be used to demonstrate anti-*Mycoplasma* antibodies. Either of these procedures is much more definitive in diagnosing primary atypical pneumonia than is the cold agglutination test.

CHAPTER 3

Latex-Fixation, Precipitin, and ASO Tests

BASIC SEROLOGICAL techniques have not changed much in the past 50 years. Recent research and development of new methods has been motivated by two requirements. The first is for tests that will quickly and easily diagnose difficult or previously undetectable diseases. The second is for tests that can be performed rapidly with a minimum of supplies and equipment. Latex-fixation and related tests have generally met these requirements. Most of these tests are prepackaged in kit form. The tests can be performed rapidly, and in some instances accuracy is greatly improved. Newer tests for rheumatoid arthritis are good examples of this. A serum specimen can be screened in 2 or 3 minutes. The older procedures required several hours to set up and were somewhat lacking in specificity.

2. Although the newer slide tests are easy to perform and interpret, the principles that govern their reactions are just as involved as the older procedures. In this chapter we shall discuss the components and theory of the reaction of latex-fixation and precipitin tests. Also we will review the antistreptolysin test with mention of a newer slide method for performing this time-consuming procedure.

7. Latex-Fixation Tests

7-1 To most laboratory technicians, when we say "latex-fixation" it is understood that we are talking about a test for rheumatoid arthritis. Latex particles may, however, be used in a variety of applications. They are used in the diagnosis of trichinosis and in the rapid slide test febrile agglutination procedures. While such test kits are supplied with charts for interpreting test results, it is desirable for you to know why the reaction occurs. With this information, quality control of the procedure is easier and the limitations of the test are easier to understand.

7-2. **Tests for Rheumatoid Arthritis.** Rhumatoid arthritis is a chronic disease of the joints. In severe cases, joints throughout the body may be affected. It is marked by inflammatory changes in the bones and tissues surrounding the joints. The disease can cause deformity and complete joint immobility in the late stages. It is now thought that the inflammation of joints in arthritis causes the body to produce an abnormal protein. This protein is of very high molecular weight (large molecule) and is referred to as a macroglobulin. Because the macroglobulin is abnormal, it initiates an immune response; the body reacts to is presence and produces an antimacroglobulin antibody. Here we have an autoimmune response. Arthritis is therefore sometimes classed as autoimmune disease. As previously defined, autoimmune diseases are peculiar conditions in which the body reacts to one of its own components or tissues and produces antibodies against that component or tissue.

7-3. Many different kinds of tests have been employed as an aid in diagnosing this disease. One of the earliest was a streptococcal agglutination test. In this test, the patient's serum was mixed with a suspension of Group A *Streptococcus* and agglutination occurred in a significant number of tests. This streptococcal agglutination test gave variable results, which probably were due to differences in the bacterial suspensions used in the tests and to the fact that the substance being detected was a very unusual type of antibody. Correlation was also noted between the presence of the antibody and a moderately elevated Todd antistreptolysin O titer.

7-4. The Rose test or some modification of the Rose test was for many years the test of choice. This test employed sheep erythrocytes sensitized by exposing them to small amounts of rabbit anti-sheep globulin. A normal control of unsensitized cells was run at the same time. When the sensitized and unsensitized cells were exposed to dilutions of serum from an arthritic patient, agglutination of a higher titer would occur in the sensitized cells than in the unsensitized cells. A significant number of arthritic patients did not give this pattern or gave variable test results.

7-5. Modifications were made to overcome the deficiencies of the Rose test. Human erythrocytes were substituted with no appreciable improvement of the test. Tannic acid was tried as a sensitizing agent. Collodion particles and other inert carriers were tried. The most recently devised tests employ latex particles approximately 0.8 micron in diameter. They are used to absorb an antigen, in this case gamma globulin, which will then react with an antibody in the patient's serum to produce observable agglutination.

7-6. Polystyrene latex particles are used in the several tests for rheumatoid arthritis currently in use. These particles are soaked in normal gamma globulin from humans or any of seven other animals. The latex particles absorb some of the gamma globulin and the excess is washed off. The coated particles are reconstituted to a specific turbidity in buffered saline. Spectrophotometers or related instruments are used to adjust the turbidity of the latex suspension so that an optimum concentration can be obtained.

7-7. The test is performed by mixing a quantity of the latex-antigen suspension with serum. If anti-gamma-globulin antibodies are present in the test specimen, the latex particles are agglutinated. While the observable reaction appears to be agglutination, the reaction that actually takes place is a precipitin reaction. The latex particles absorb a soluble antigen. Reactions between soluble antigens and antibodies result in precipitation or the formation of soluble antigen-antibody complexes. The gamma globulin (antigen) used in this test is soluble. We speak of the test as a *latex-fixation* because the antigen (γ-globulin) is fixed (absorbed) by the latex carrier particle.

7-8. A modification of the basic, simple latex-fixation test is the eosin latex-fixation test of Singer and Plotz. Although this test was designed to detect rheumatoid arthritis, it can be used in the diagnosis of a variety of collagen and tissue destruction diseases. Among the diseases giving a positive test are liver diseases, lupus erythematosus, hypergammaglobulinemia, sarcoidosis, and nonrheumatoid arthritis. Therefore, you will occassionally get a request to perform this test on nonarthritic patients. Perform the test and report the results in the same manner that you would if the specimen were from a rheumatoid patient. The significance of a positive test in the diseases mentioned above is determined by the physician on the basis of the clinical aspects of the patient's condition.

7-9. Latex-fixation tests performed in test tubes are more accurate than slide test screening procedures. It is important that you run appropriate controls with each batch of tests, especially since you will be using a stored preserved antigen which can become slightly lumpy after a period of storage. While slide screening procedures are adequate in most instances, there will be occasions, such as in observing the effect of therapy, when precise titrations are required. The tube test will then be the method of choice.

7-10. **Other Tests Using Latex Particles.** Latex particles have been used as antigen carriers in a varity of commercially developed diagnostic test procedures. Soluble antigens are applied directly to the latex particles for absorption. Particulate antigens, such as suspensions of bacteria or macerated worms, are reduced to fine suspensions. These suspensions are prepared using any of several techniques, such as grinding, ultrasonics, or lysing. Commercial manufacturers have found ultrasonics to be the most satisfactory way of producing a smooth even suspension.

7-11. Latex particles have also been used in tests to detect C-reactive protein. This protein appears in the blood in acute pneumococcal pneumonia. It has also been isolated in many other diseases and infections including myocardial infarction, streptococcal and staphylococcal infections, Hodgkin's disease, cirrhosis of the liver, and certain cancers. C-reactive protein can be identified by its ability to precipitate the somatic C-polysaccharide of rough stains of pneumococcus. Electrophoretically, the protein has properties similar to β and γ-globulin. Its sedimentation constant is higher than serum γ-globulin. It has not been classified as an antibody.

7-12. To detect C-reactive protein, you use antiserum obtained by injecting rabbits with purified C-reactive protein. You use Latex particles coated with the antiserum and mix them with the patient's serum. Visible agglutination occurs rapidly if C-reactive protein is present. In most instances, you run this test as a simple slide-test screening procedure.

7-13. Manufacturers prepare febrile and other bacterial agglutination test antigens by soaking latex particles in extracts of broth cultures in which the desired microorganisms were cultured. The excess antigen is washed off and the latex suspension is diluted to desired turbidity. A drop or two of the commercially prepared diluted latex suspension plus a drop or two of the patient's serum provide an excellent rapid screening test; considerably less time is required than in the older tube test.

7-14. Lupus erythematosus, a disease of connective tissue, causes the body of those affected to produce antibodies to desoxyribonucleic acid (DNA). You can detect these antibodies with DNA coated latex particles. The latex particles are carriers for the DNA antigen, Run this test as a screening procedure along with other tests for lupus, such as the L.E. prep.

7-15. Trichinosis, a nematode infestation of muscle tissue, is caused by eating raw or insufficiently cooked pork. The worm's presence in muscle tissue elicits an immune response. You can detect antibodies with latex particles which carry antigens of the trichina worm. The commercially prepared product is a macerated extract of the worm absorbed onto latex particles. Mix a drop or two of the latex-antigen suspension with a drop or two of the patient's serum on a slide to make a simple, efficient screening test.

8. Precipitin Tests

8-1. The precipitin reaction was first observed in 1897 and is one of the basic methods used to observe antigen-antibody reactions. There are a great many applications where precipitin reactions can be of use. These include the estimation of antigens or antibodies in solution, in tissues, and in individual cells. The techniques devised to make these reactions visible include such diverse methods as diffusion, use of gel media, fluorescence, X-ray opacity, radioactive isotope tagging, or coupling the antigen or antibody with an enzyme. Most often a finely flocculent precipitate indicates a positive reaction. However, in some precipitin reactions, the reactions are not directly observable and one of the methods mentioned above must be used to show that a reaction has taken place.

8-2. In precipitin tests, the antigen is serially diluted and is mixed with a constant volume of antiserum. The amount of antiserum usually required is 1 ml. or more. This is a relatively large amount of antiserum when compared to the amount required in agglutination tests. A dozen or more agglutination tests can be performed using this same amount of antiserum. The agglutination reaction is more sensitive than the precipitin reaction. A few antibodies are capable of causing visible clumping of a particulate antigen such as blood cells. The precipitin reaction (of soluble antigen-antibody) is less easily seen. Many tests have been designed to overcome this deficiency. One way of doing this is to coat cells with an antigen which then reacts with a smaller amount of antiserum. Attaching the antigen or antibody to an inorganic carrier particle (e.g. latex particle) is the most frequent way of making the reaction visible.

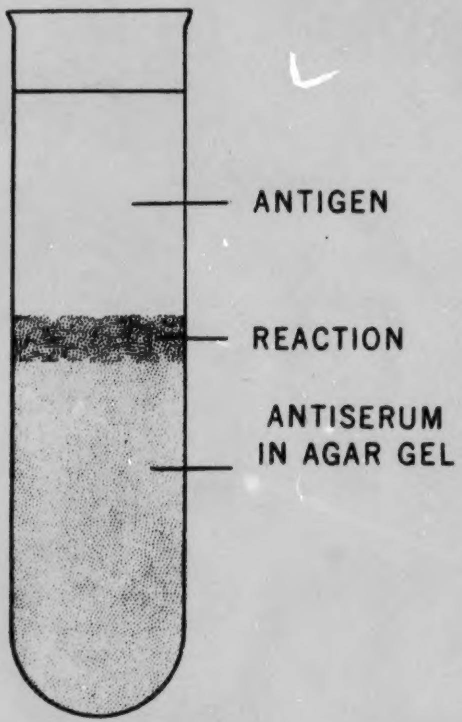

Figure 13. Simple diffusion technique (tube).

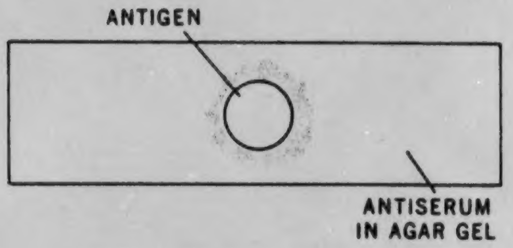

Figure 14. Simple diffusion technique (slide).

8-3. The precipitin reaction is not widely used in most USAF serological laboratories. It is, however, employed in the frequently performed VDRL test for syphilis. Special techniques have recently become available to those laboratories which need highly refined methods for identifying small amounts of antigen-antibody products. There are two of these special methods not previously mentioned in this volume which are of increasing interest to the serologist because they make clearly visible certain reactions that were not previously well defined. These two methods are simple diffusion and the Ouchterlony double diffusion technique.

8-4. **Simple Diffusion.** Simple diffusion is a very simple procedure useful in semiquantitative analyses. The test involves putting a volume of a solution containing the antiserum into a test tube (or capillary tube) and carefully overlaying it with a quantity of antigen. In these tests the antigen and antiserum are solutions and require very delicate pipetting in order to overlay the antiserum without mixing the two. For a simple modification of this procedure, mix the antiserum in a 0.5-percent to 2-percent agar gel and allow the gel to solidify. You can then easily place the antigen in contact with the antiserum without mixing. The reaction takes place at the interface (point of contact) of the antigen and antiserum. The simple diffusion technique is shown in figure 13. Simple diffusion is "simple" because the procedure requires a single diffusion step.

8-5. A second method of simple diffusion is to pour the antiserum-agar gel mixture onto a slide or Petri dish and allow it to solidify. Cut wells into the agar, and fill the wells with antigen suspension. After incubation at room temperature, examine the preparation for the formation of rings of precipitate

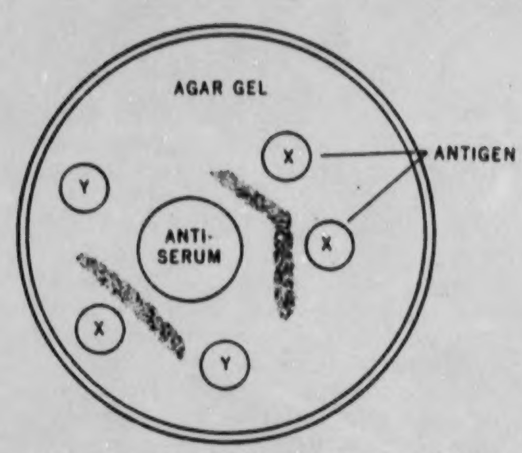

Figure 15. Double diffusion technique (Ouchterlony).

around the wells. This technique is demonstrated in figure 14.

8-6. **The Ouchterlony Technique.** This technique involves double diffusion as opposed to the simple diffusion of the previous tests. Both antigen and antiserum are allowed to diffuse into an agar gel medium. To do this, pour a pure agar suspension into a Petri dish and allow it to solidify in the pattern shown in figure 15. Cut wells into the agar and fill the outside wells with antigen and the central well with antiserum. Keep the dish covered and moist, and allow it to incubate. Reaction is indicated by the formation of lines of precipitate between the antigen and its antiserum. The reaction is illustrated in figure 15. In this illustration antiserum (anti-X) has been placed in the center well and antigens X and Y in the outer wells. This technique is very sensitive and is frequently used to identify antigens or antibodies in mixtures.

9. Antistreptolysin O

9-1. When certain strains of *Streptococcus* organisms infect the body they produce a substance which reacts enzymatically with red and white blood cells, thus destroying them. Group A strains of *Streptococcus* are the most frequent producers of this substance. It can also be produced by certain Group C and G organisms. This hemolytic substance (streptolysin) is really composed of two hemolysins. The first, streptolysin S, is an oxygen-stabile, nonantigenic lipoprotein. Due to its lack of antigenic activity, there is no practical way to measure it with available laboratory tests. The second hemolysin, streptolysin O, is oxygen-labile and a very good antigen. Procedures are available for measuring this hemolysin and the test can be performed in laboratories of any size.

9-2. When the body is infected with one of the organisms which produce streptolysin, an immune response is initiated by the presence of streptolysin O. The body responds by producing antistreptolysin O antibodies. These antistreptolysin O antibodies are capable of neutralizing streptolysin O and rendering it incapable of hemolysing cells. This aspect of the streptolysin O and antistreptolysin reaction is made use of in the test we commonly call the antistreptolysin O test.

9-3. **The ASO Titration.** In the antistreptolysin test, you expose a serial dilution of suspected serum (inactivated or not) to a standardized streptolysin O solution. After a period of incubation, add human red cells to each tube of the dilution. If the patient has developed antistreptolysin O antibodies the standardized streptolysin O solution will be neutralized and the blood cells will *not* be hemolyzed. If no antibodies are present, the red blood cells will be hemolyzed by free streptolysin O.

9-4. Report the last dilution (tube) showing no hemolysis as the titer of the test. Test results are reported as Todd units (a titer of 100 equals 100 Todd units, etc.) Highest normal titers are seen in school age children. Their normal titers can be as high as 333 Todd units. Normal healthy adults usually do not have titers higher than 200 Todd units. This difference in normal values is primarily due to children having more streptococcal infections than adults.

9-5. Antistreptolysin titers are particularly high in rheumatic fever and glomerulonephritis. Most acute rheumatic fever patients have titers of 300 to 1500 Todd units. A small percentage may, however, exhibit normal ASO titers. The titers remain high for as much as 6 months in this disease. In suspected rheumatic fever and glomerulonephritis, tests are run in series several weeks apart. An increasing titer is more indicative of rheumatic fever than a single high titer.

9-6. An increased ASO titer can be seen in many infections and diseases. Included among these are chronic sore throat, severe acne, pneumococcal pneumonia, and rheumatoid arthritis. The titer seen in these diseases is not as high nor does it persist as long as the elevated titer in rheumatic fever. If the patient with rheumatic fever is being treated with antibiotics such as penicillin or aureomycin, a decrease in titer may occur from the beginning of treatment. These antibiotics can inhibit the in vivo production of streptolysin O. This inhibition lessens the immune response, resulting in lowered titers.

9-7. Quality control of the ASO test does not differ from that of any other serological procedure. There are two common sources of error; guard against these to insure accurate tests. The first is to be sure to use fresh cells if possible. Wash these cells thoroughly to remove any residual antistreptolysin O antibodies that the donor may have possessed. The second thing to watch is the streptolysin O reagent used in the test. As stated before, streptolysin O is oxygen-labile. If left standing at room temperature or if reconstituted and put into the refrigerator, the

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streptolysin O reagent becomes inactivated. It must be used *immediately* after reconstitution. Improper handling of this reagent is the most common source of error in the ASO test.

9-8. **ASO Latex Test.** A procedure using a preserved antigen has recently become available. The preserved antigen is absorbed onto a latex particle carrier. This test is available as a prepackaged kit. It is primarily intended as a screening test and, according to the manufacturer, is best used to separate those patients with normal titers from those with elevated titers. Do not forget that there are certain conditions and diseases where a decreased titer is expected. Consequently, the screening test is not a complete substitute for the standard ASO test.

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CHAPTER 4

Serological Tests for Syphilis

THE SYPHILIS organism was discovered in 1905 by Fritz Schaudinn and Paul Hoffman. Schaudinn was a Prussian protozoologist who may have considered his discovery a protozoan. He called it *Spirochaeta pallida,* and it was eventually renamed *Treponema pallidum.* Dr. Hoffman was professor of dermatology at the University of Bonn. He worked closely with Schaudinn and was able to establish the presence of the causative organism in the lymph glands of patients with syphilis.

2. It was the famous German chemist Paul Ehrlich who developed the first effective treatment for syphilis. A pioneer in chemotherapy, Ehrlich developed and tested over 600 arsenic compounds with regard to their capacity to kill various organisms. One of these compounds, known as No. 606, was reported by one of Ehrlich's assistants to be ineffective in its action on the syphilis spirochete. Fortunately, Ehrlich hired a new assistant, Sahachiro Hata, who retested compound 606. He found that a mistake had been made and that 606 was indeed effective in the treatment of syphilis. Compound 606 was named *Salversan,* and it was applied clinically in 1911. Ehrlich modified *Salversan* several times and was awarded the Nobel Prize for his work. (*Salversan* is now called neoarsphenamine.)

3. The first useful laboratory test for syphilis was developed by August von Wassermann and Albert Neisser, the discoverer of the gonococcus, and their associates. Although *Spirochaeta pallida* was discovered in 1905, it had not yet been cultured. Therefore, instead of the organism itself, Wassermann used an aqueous extract of the liver from a syphilitic foetus. The test was described as a complement fixation test, although it did not really involve a complement fixing reaction. In 1907 an investigator at the Institute in Paris showed that extracts of normal liver gave the same results, i.e., patients with syphilis reacted positively, and most normal controls tested negative. With several modifications, the test using liver, and later heart extract, continued to be of diagnostic value. The Wassermann test formed the basis for many of the tests which will be described in this chapter.

4. We will begin this chapter with a discussion of some general aspects of syphilis and the serology of syphilis. Following this we will describe a number of tests for syphilis, including fluorescent antibody techniques. Pay special attention to the variety of techniques and the specificity (sensitivity) of each of the various tests. Few areas of serology are as confusing to the practitioner as the many tests used to diagnose syphilis. As a laboratory technician you have the responsibility to advise physicians on the availability and specificity of the tests performed in your laboratory; and, what is more important, it is your job to perform each test without personal modification or "shortcuts." If you conscientiously adhere to *standard* procedures, your results can be correctly interpreted. In other words, tests for syphilis are *not* all alike. Medical and legal requirements often dictate that distinctions be made among the various tests for syphilis, and it is in this area that your knowledge is both practical and valuable.

10. Syphilis and the Serology of Syphilis

10-1. We will now turn our attention to the serology of syphilis, with a few paragraphs about the disease itself. Also included are some facts about serologically related infections.

10-2. Many interesting things have been written about syphilis, partly because syphilis made an impact throughout modern history, and also because this is a venereal disease with some rather frightening symptoms. It leads to infiltration and destruction of brain tissue, and this results in disability, insanity, and death. Evidence suggests that syphilis first appeared about 500 years ago, though medical practices and methods of reporting may have obscured the prevalence of the disease at earlier times in history. Many historians believe that syphilis was introduced into Europe by members of Columbus' crew when they returned to Europe from the West Indies. Incidentally, it is suggested that Columbus himself may have suffered from syphilis. During his third voyage in 1498, Columbus was described as having a "severe attack of gout." He developed mental symptoms, and finally after his last voyage in 1504, had to be carried ashore. Columbus had all of the symptoms of terminal syphilis.

10-3. In 1496 syphilis spread throughout Europe and was known as "The Great Pox." Affected individuals were quarantined and in many cases banished to islands or colonies similar to leper colonies. By the early 1500's syphilis had spread to England and eastward to China. You may recall from popular accounts that Italians called syphilis the French or Spanish disease, the English called it the French disease, and French called it the Spanish disease.

10-4. The term *syphilis* was coined by Fracastorius, the famous Italian physician, when he wrote a poem about the disease in 1530. Syphilis was a shepherd in the poem who was stricken with this dreadful disease by Apollo. Perhaps Fracastorius contributed to the prevalent belief that syphilis represented a punishment for sin and was not to be approached with quite the same "objectivity" as nonvenereal diseases.

10-5. Today, two forms of syphilis are recognized. The first is *venereal syphilis* and is worldwide in occurrence. ("Venereal" relates to sexual intercourse.) The second type of syphilis is *endemic syphilis* and is confined to certain parts of the world outside of the United States.

10-6. **Venereal Syphilis.** Characterized clinically by a primary lesion, a secondary eruption involving the skin and mucous membranes, and finally after what is often a long latent period, lesions of the skin, internal organs, bones, and central nervous system. The first lesion, often called the *primary* lesion or chancre appears about 3 weeks after infection. Needless to say, diagnosis is left to a physician, particularly since other disease processes may involve a lesion and also because treatment is necessary. One sometimes hears of individuals, often medics, who are not physicians, who attempt to treat themselves or their acquaintances to avoid possible administrative or social consequences. This can be dangerous because inadequate treatment in the primary stage will mask symptoms and lead to the more serious secondary stage. Secondary manifestations always appear in untreated cases. These can appear within weeks or as long as 12 months after infection. Secondary symptoms may include lesions of the eye and central nervous system as well as lesions of the skin. Secondary symptoms are followed by a period of latency, and sometimes tertiary or late disabling manifestations do not occur at all. More often, late manifestations shorten life and disable the victim in various ways.

10-7. As you know, the infectious agent is *Treponema pallidum*, a spirochete. Man is the only reservoir, and infection is by means of exudates from lesions of the skin or mucous membranes. Indirect contact is considered of little significance because *Treponema pallidum* does not survive under conditions of unfavorable moisture, temperature, and the like. It is possible to contract the disease while handling body fluids in the laboratory where such fluids are taken from areas of active early lesions. Serum is not *usually* considered a potential source of infection to the laboratory technician, but it could be. Prenatal infection may occur after the fourth month of pregnancy through placental transfer. Transmission of syphilis by blood transfusion is unlikely because of the storage temperature of blood and the survival time of *Treponema pallidum* under storage conditions. However, as you know from your reading earlier in this course, blood donors are screened to preclude the use of blood from a donor with syphilis.

10-8. There is no natural immunity to either venereal syphilis or endemic syphilis. Infection does lead to a gradually developing resistance to strains which are closely related, but there is little protection afforded to heterologous strains. The control of syphilis from an epidemiology standpoint is a matter for the public health section of the hospital, and this involves the laboratory only indirectly. Often, coordination of laboratory data such as positive serological tests may be of value to the hospital Registrar or Military Public Health officials. You must be careful in drawing conclusions from laboratory data alone, however.

10-9. **Endemic or Nonvenereal Syphilis.** This type of syphilis is acute and of limited geographical distribution. It is transmitted by direct and indirect contact with infectious lesions, often spread by the use of common eating and drinking utensils. The infectious agent is *Treponema pallidum*. Thus, you see that unless a strain different from that which causes venereal syphilis is involved, endemic syphilis is etiologically the same as venereal syphilis. But from the standpoint of the mode of transmission and geographical occurrence, the distinction between venereal and nonvenereal syphilis is valid and useful, especially in efforts to control communicable diseases. Further, unlike venereal disease, nervous and cardiovascular system involvement is rare with endemic syphilis. Fatalities are also rare in cases of nonvenereal syphilis. It is common in Africa, the Balkans, and along the Mediterranean. This disease also has various synonyms, including *bejel, dichuchwa, njovera, sibbens,* and *radesyke.*

10-10. **Serologically Related Infections.** There are several spirochete diseases which give false results in serological tests used to detect the syphilis antibody. Among these diseases are yaws, pinta, and rat-bite fever. In addition, positive serological tests have been reported in a variety of etiologically unrelated disorders, including disseminated lupus erythematosis.

10-11. *Yaws.* One of the diseases which presents a serological picture easily confused with that of *T. pallidum* is *yaws*, an acute and chronic nonvenereal tropical and subtropical disease caused by *Treponema pertenue*. Yaws resembles syphilis but does not involve the viscera or central nervous system. Neither does it occur congenitally. (Congenital transmission of *nonvenereal* syphilis is described as "rare.") Yaws is predominantly a childhood disease but often does occur in older people, especially males. It is common in Southeast Asia, in Africa, the Philippines, throughout the South Pacific Islands, in the Caribbean, and in South America. Serological tests for syphilis present a confusing picture in these areas because of the incidence of antibodies to *T. pertenue*. Like syphilis, there is no evidence of natural or racial resistance, except that infection results in immunity to homologous strains.

10-12. *Pinta.* This is an acute as well as a chronic infection by *Treponema carateum* which causes some of the serological tests for syphilis to give a positive reaction. Serological tests for syphilis usually become reactive during the secondary rash of this disease. It is a common disorder in the tropics and subtropics but is not limited to these areas. It is also common in Mexico and certain countries of South America, especially Venezuela, Colombia, and Ecuador. In addition, pinta is found in North Africa, the Middle East, and in India and the Philippines.

10-13. *Rat-bite fever.* The term *rat-bite fever* is nonspecific. It refers to two different diseases transmitted by bites of infected rats. One of the diseases is known as *Streptobacillus moniliformis* disease and the other as *Spirillum minus* disease. It is the latter which presents a syphilis-like serological pattern and, to some extent, a clinical picture similar to syphilis. The disease is sometimes called sodoku, or sporadic rat-bite fever. The correct name of the spirochete that causes *Spirillum minus* disease is *Spirocheta morsus muris*. It is common in Japan and in the Far East. The important thing for you to realize is that there are many relatively common diseases, including the three just described (yaws, pinta, and rat-bite fever) which cause false positive serological tests for syphilis.

10-14. **Antibody Development.** Infection with *T. pallidum* causes the body to produce both nonspecific reagin and specific antibodies. Reagin is produced by most patients infected with *T. pallidum*, but is not a specific antibody to the organism and is not measured with a specific antigen. It has been suggested that reagin is produced by the body as a secondary response to tissue damage. Reagin consists of a high molecular weight antibody component, and a relatively low molecular weight antibody fraction. The low molecular weight antibody takes part in flocculation reactions, and the high molecular weight antibody reacts in complement fixation tests. Antigens used to detect reagin antibodies are lipid complexes, not treponemal antigens. Both living and killed Nichols strains of *T. pallidum* and extracts of Nichols and avirulent Reiter strains of the organism are used to detect specific antibodies.

10-15. There is usually a measurable antibody response from 1 to 3 weeks after appearance of the primary chancre. Exacting interpretation depends upon the particular test and clinical considerations. In general, it is not possible to detect syphilis antibodies within the first few weeks of primary infection. Probability of detecting the presence of syphilis antibodies after this initial period varies with the principle of the test used. As you know, there are many tests for syphilis. The term *serological test for syphilis (STS)* has no real specific meaning, though it is used by some serologists to indicate those tests which detect reagin, i.e., tests which employ nontreponemal antigens. In Section 11 we will discuss some of the tests which are used throughout the Air Force. Test selection by the physician often depends upon which tests are available and the specificity of the tests. The use of multiple tests with hope of a consensus is not a logical application of serological principles. The reasoning that two serodiagnostic tests are better than one, and three or more are the ultimate in serodiagnosis is no longer sound.

10-16. Although serologic tests for syphilis are not absolutely specific and some sera are reactive in one test and nonreactive in another, analyses of conflicting serologic results in terms of diagnosis or prognosis are only within the province of the physician. Valid serologic test results are *your* responsibility and are obtained only when you (a) use standardized reagents and adequate controls, (b) adhere strictly to standardized techniques, and (c) report the results as specified for each procedure.

11. Serological Tests for Syphilis[1]

11-1. In this section we will discuss some of the most important laboratory tests for syphilis. It is not possible to state in this course which tests should be used or the desirable order of tests for all clinical cases. In general, flocculation tests are used for screening and may be of two types—qualitative and quantitative. Qualitative tests are reported as reactive (R), weakly reactive (W), and nonreactive (N). Quantative results represent a serial dilution of the patient's serum. Results of a quantitative test are reported in terms of the highest dilution of serum that produces a reactive (not weakly reactive) result. Quantitative serological tests help the physician in evaluating treatment. If the titer is established before treatment, a falling titer or failure of the titer to rise will suggest satisfactory progress. An increasing titer of the syphilis antibody usually indicates an active disease process. A single high titer by itself does not, of course, always mean an active infection or even an infection of *T. pallidum* at all.

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[1] Information contained in this section is based on material prepared by the U.S. Public Health Service and published by the U.S. Government.

11-2. Specific antibody tests such as the fluorescent treponemal antibody absorption (FTA-ABS) are used routinely in some facilities, whereas other laboratories may reserve specific antibody tests for problem cases or cases in which history, clinical findings, dark-field examinations, and routine serologies are not diagnostic.

11-3. **Quality Control.** There are many factors, such as equipment, reagents, time periods, volumes, and temperatures which influence test performances. In addition, inter- and intra-laboratory checks are strongly recommended to assure adequacy of test performance. These include the daily use of controls of graded reactively, periodic check readings to maintain uniform reading levels among the laboratory personnel, and comparison of results obtained on control serums with those of a reference laboratory.

11-4. *Serum controls.* Include control specimens of graded reactivity each time you perform serologic testing. For the nontreponemal flocculation tests with serum and spinal fluid, the antigen suspension to be used each day is tested for proper reactivity by checking it against control specimens of known reactivity. The results obtained with the controls should reproduce a previously established reactivity pattern. If the results are not acceptable, delay routine testing until you have established better reactivity (by preparing another antigen suspension, correcting room temperature, adjusting equipment, etc.). For the FTA-ABS test, the control specimens are included in the test run. If the pattern of reactivity is not acceptable, results of the tests on individual specimens are considered invalid and are not reported.

11-5. Control specimens of graded reactivity for nontreponemal and treponemal test procedures are available from commercial sources or may be prepared from specimens pooled after testing. Reactive serum of high titer may be used to prepare spinal fluid controls. A pattern of reactivity should be established for each new lot of control serum prepared in the laboratory, or confirmed for each new lot of control serum obtained from a commercial source by comparing the new control serum with standard control serum. Serum samples to be submitted to other laboratories for syphilis serology proficiency testing studies may be prepared in a manner similar to that for control specimens of graded reactivity.

11-6. Detailed instructions on the preparation of serum controls for nontreponemal antigen tests and for the FTA-ABS test may be found in *Manual of*

Table 11
Delivery needle specifications

Test	Reagent	Needle gage	Size drop required (ml)	No. drops delivered per ml of reagent
RPR (circle) Card	Antigen suspension	20	0.017 or 1/60	60 ± 2
USR	Antigen suspension	18	0.022 or 1/45	45 ± 1
VDRL (qualitative)	Antigen suspension	18	0.017 or 1/60	60 ± 2
VDRL (quantitative)	Antigen suspension	19	0.014 or 1/75	75 ± 2
VDRL	0.9-percent saline	23	0.010 or 1/100	100 ± 2
VDRL	Sensitized antigen suspension	21 or 22	0.010 or 1/100	100 ± 2

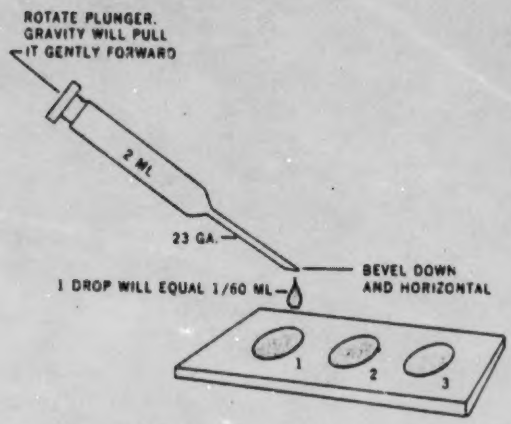
ROTATE PLUNGER. GRAVITY WILL PULL IT GENTLY FORWARD
2 ML
23 GA.
BEVEL DOWN AND HORIZONTAL
1 DROP WILL EQUAL 1/60 ML
1
2
3

Figure 16. Dispensing needle for slide flocculation tests.

Tests for Syphilis, 1969, published by the U.S. Public Health Service.[2]

11-7. *Equipment.* Keep all equipment clean and in good working condition. Check water bath temperatures during the day each time that they are used. Check refrigerator temperatures daily with a thermometer placed in the part of the refrigerator occupied by the test racks. Also be sure to notice the temperature when the refrigerator is first opened in the morning if complement-fixation tests (16- to 18-hour fixation) are stored within.

11-8. Check the speed of shaking and rotating machines each time they are used, and do not tolerate a marked variation from prescribed speeds.

11-9. Centrifuges should be equipped with tachometers so that speed may be checked and controlled. Clean the inside of centrifuges occasionally to prevent dust particles from being blown into specimens.

11-10. Check automatic pipetting machines daily for correct volume delivery. Should readjustment be found necessary, collect and measure a volume of 25 or 50 deliveries in a certified graduated cylinder.

11-11. *Calibration of dispensing needles.* To calibrate a dispensing needle for use in slide flocculation tests, file a deep nick in a hypodermic needle just above the bevel. Break the needle point off with pliers. Using a 1-ml. or 2-ml. syringe containing the material to be dispensed, check the needle delivery by counting the number of drops in 1 ml. of reagent. Allow the drops to fall freely from the tip of the needle while you hold the needle and syringe *perpendicular* to the tabletop. Specifications for delivery are outlined in table 11. If a needle does *not* meet the above specifications, adjust it to deliver the correct volume before you use it. An alternative method of emulsion delivery is suggested in AFM 160-47. In this case, use a 23-gage hypodermic needle with the bevel intact and parallel to the slide, as shown in figure 16.

11-12. If too few drops per milliliter are delivered by the needle, the opening of the tip is too small. Ream it out with a sharp-pointed instrument, such as the sharpened end of a triangular file. If too many drops per milliliter are delivered by the needle, the opening of the tip is too large. Adjust it by mashing the sides together slightly or by filing the edges of the needle inward. Once they are calibrated, protect the tips of needles against dropping on the floor, the sink, or to the bottom of bottles. Check the needles *each day* before using, and adjust them if necessary. Clean needles and syringes by rinsing with water, alcohol, and acetone. Remove the needle from the syringe after cleaning.

11-13. *Glassware.* Use only chemically clean glassware in the serology laboratory. Tubes and pipettes from which protein solutions have not been completely removed will acquire a brown film. You can usually remove this film by putting the tubes and pipettes in sulfuric-acid-dichromate cleaning solution for at least 4 hours. Whether you use alkaline or acid solutions for cleaning, rinse the glassware thoroughly to remove all traces of cleaning solutions. It is recommended that you make the final rinse in distilled water. Daily spot testing of glassware with indicator solutions will insure against the release of chemically contaminated glassware to the testing laboratory. The use of disposable glassware is desirable. However, you must carefully check even disposable glassware to be sure it is spotless and free of packing materials, such as bits of straw or paper.

11-14. Glassware used for each test should meet the recommended specifications. Use pipettes and cylinders of appropriate sizes and graduations for measuring reagents and specimens. Discard and replace any tubes, slides, or pipettes that become etched or scratched to a degree that will interfere with test readings.

11-15. *Reagent control.* It is your responsibility to insure that reagents are of good quality and standard reactivity. Chemicals and distilled water that you use should be of high quality, and you should prepare solutions according to directions specified in each technique. Check test each new lot of cardiolipin antigen or antigen suspension (RPR card and USR), and antigen, sorbent, and conjugate for the FTA-ABS test in parallel with a standard reagent to verify that it is of standard reactivity. Perform parallel testing on more than one testing day, by using different specimens of graded reactivity for each test period. Keep a permanent record of the results of all check testing. You can obtain individual specimens of graded reactivity for check testing by selecting specimens from daily test runs and storing them in the freezer. Use fresh nonreactive specimens from routine test runs as nonreactive controls.

11-16. Distilled water of poor quality may result from failure to clean the still or storage container as frequently as needed. The kind of tap water used and the number of hours per day that the still is in operation are factors determining the frequency of cleaning. Periodic checks of pH and conductivity serve as reliable indications of the purity of distilled water. If stored, distilled water should be placed in hard-glass or plastic containers that are tightly stoppered to avoid changes due to ion transfer from the glass and absorption of gases present in the laboratory. The use of freshly distilled water or water for injection is preferred.

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[2] For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington D.C. 20402—Price $3.

11-17. Prepare normal saline from sodium chloride dried in the hot-air oven for 30 minutes at 160° to 180° C. to remove absorbed moisture. Heating at higher temperatures should be avoided since it may result in decomposition of the salt. Weigh the sodium chloride into prescribed amounts and store in corked test tubes to avoid daily weighing. Dissolve salts in distilled water. Shake the solution thoroughly to assure complete mixing. If you store the solution, place it in hardglass, tightly stoppered containers.

11-18. *Temperature.* Serologic tests for syphilis are influenced to varying degrees by the temperature at which they are performed. Some tests are performed at prescribed water bath or refrigerator temperatures. For uniformity of results, other tests should be performed at room temperatures between 73° and 85° F. (23° and 29° C.).

11-19. *Contamination.* Grossly contaminated serum specimens or spinal fluids are unsatisfactory for serological testing. The effects of random bacterial contamination on serologic results are not always predictable. Although spinal fluid is usually drawn with reasonable attention to sterility, many fluids mailed to central testing laboratories, especially during the warm months of the year, show evidence of gross bacterial contamination on arrival. Removal of bacteria from contaminated spinal fluids by centrifuging or filtering is ineffective since some of the products of bacterial metabolism are soluble.

11-20. The use of Merthiolate ® as a bacteriostatic agent for spinal fluid preservation has been suggested by the U.S. Public Health Service. This compound (sodium ethyl-mercurithiosalicylate) suppresses bacterial growth without interfering with the mechanisms of the usual serologic tests for syphilis either through chemical action or the introduction of a significant dilution factor. Furthermore, its presence does not affect the results obtained with the turbidimetric methods for determining total proteins in spinal fluids.

11-21. **Cardiolipin Microflocculation Test (CMF)—VDRL.**[3] The cardiolipin microfloccutation test detects the presence of syphilitic reagin by means of a reaction between the reagin and a standard antigen. The antigen used in this test is composed of cardiolipids and lecithin which have been extracted from beef heart and purified. Cholesterol is added to the alcoholic mixture of the cardiolipids and lecithin for the purpose of increasing the antigen's effective reacting surface. The antigen and buffered diluent are supplied through Air Force supply channels; pH range of the diluent must be between 5.9 and 6.1.

11-22. Syphilitic reagin is capable of producing changes in the dispersion of the cardiolipid particles which result in visible flocculation. It is believed by some authorities that there is a distinct serologic relationship between some of the antigens in *Treponema pallidum* and the beef heart lipids.

11-23. Two flocculation procedures are described in AFM 160-47. The first is a rapid slide test, yielding qualitative results, which should be employed as a screening procedure. If any reaction is observed in the qualitative test, the serum should be serially diluted and checked in the quantitative test which is the second flocculation procedure to be discussed.

11-24. Heat clear serum obtained by centrifuging whole clotted blood for 30 minutes in a water bath at 56° C. before testing to inactivate the complement and thereby activate the serum. Examine all sera immediately after removing them from the water bath. If any of them contain small particles the specimen should be clarified by centrifuging again. Sera to be tested more than 4 hours after heating should be reheated for 10 minutes in the water bath at 56° C.

11-25. *Qualitative CMF.* The qualitative cardiolipin microflocculation test is a rapid slide screening procedure employed to detect the presence of syphilitic reagin in patients' sera. In order to determine the titer of the reagin, however, the qualitative procedure is followed by the quantitative procedure.

11-26. *Quantitative CMF.* The quantitative cardiolipin microflocculation test is performed on all specimens yielding weakly reactive or reactive results in the qualitative test. The reagents and apparatus employed in the qualitative test are used in the quantitative procedure as well. All of the preliminary testing conducted prior to the qualitative test should also precede the quantitative procedure. Quantitative tests are performed with serial dilutions of serum in normal saline. Each dilution is treated as an individual serum.

11-27. *Interpretation of CMF tests.* Patients with syphilis demonstrate a wide range of variability in the amount of reagin present in their sera. As a result, it is impossible to say that a certain level of reagin is diagnostic for the disease. A few general statements can be made, however, concerning changes in reagin content in the same individual during the course of infection.

11-28. There is no relation between the amount of reagin in the blood serum during a syphilitic

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The term *VDRL*, which stands for Venereal Disease Research Laboratory, is generally used synonymously with CMF. The antigens are identical.

infection and the severity of the disease. On the average, reagin does not become demonstrable until 6 to 8 weeks after infection. Occasionally the CMF tests for syphilis become positive by the fourth of fifth week following infection with *Treponema pallidum*. At the other extreme, in occasional instances, reagin may not become demonstrable in the blood until 12 weeks after infection.

11-29. Once reagin begins to form, it usually increases rapidly until it reaches a peak during the secondary stage. After the secondary stage has ended, as a rule, the amount of reagin in the blood decreases until it reaches a fairly stable level during the late course of the disease.

11-30. *Sources of error.* There are a number of possible sources of error inherent in CMF tests. Some techniques for preventing many of these errors are listed here:

a. Check interval timers and automatic timers on rotators for accuracy. Check the speed of the electrical rotators daily with each run.

b. Use glass tubes and mixing bottles of the exact size specified in the directions.

c. Never prepare antigen emulsions of less than the minimum quantity stipulated in the instructions. Mixing of half quantities, for example, has proved unsatisfactory.

d. Prozone reactions occur in any seroligic test. In such cases a strongly reactive serum may show a weak or a typical reaction in undiluted serum. Therefore, titration of all reactors is recommended. A completely negative reaction due to prozone phenomena in very strongly positive sera is extremely rare.

e. Test each new lot of reagent in parallel with one that is being used before the new lot of reagent is placed in routine use. This procedure is recommended regardless of the source from which the new reagent is obtained.

f. Use only reagents that are chemically pure and free from bacterial contamination. The glassware that you use in serologic tests for syphilis must be scrupulously clean.

11-31. **Rapid Plasma Reagin (RPR) Test.** Many laboratories are now using the RPR card test *as a screening procedure.* The emulsion is a carbon-containing, specially formulated cardiolipin antigen, and the flocculation is observed on plastic coated cards. The RPR antigen should be checked with controls prior to each series of tests. The entire test kit, including controls, is available commercially. Instructions and precautions are supplied by the manufacturer.

11-32. **Plasma Crit (PCT) Test.** This test is acceptable as a screening procedure. Like the RPR test, it is not bo be used for final diagnosis or control of syphilis treatment. The antigen is prepared from VDRL antigen, and is identical to that used in the unheated serum reagin (USR) test described in current laboratory manuals. Plasma, usually directly from a capillary tube, is reacted with 1 drop (0.01) ml. of PCT antigen suspension on a slide. Results are read at 100 × magnification immediately after rotation at 180 r.p.m. for 4 minutes. It is important that you use exactly prescribed volumes of reagents in this test.

11-33. **Complement Fixation Tests.** Complement becomes bound or "fixed" in a wide variety of other antigen-antibody reactions. This process, leading to the removal of free complement from the system, is referred to as complement fixation. It provides a sensitive means of detecting antigen-antibody reactions. In order to determine whether complement has been removed from a system, sensitized sheep erythrocytes, which are used as an indicator, are added to the system. If hemolysis occurs, complement is present and has not been bound. If no hemolysis occurs, complement has been bound and is not available for the hemolysis of cells.

11-34. The binding or fixing of complement has found practical application in the detection of syphilitic reagin. This test is performed by adding standard cardiolipin antigen plus guinea pig serum complement to an unknown serum. After allowing this system to react for a short time, the sensitized sheep cells (sheep cells plus suitable hemolysin) are added. If hemolysis occurs, free complement is present and the unknown serum contains no reagin. If hemolysis does not develop, complement has been bound by the antigen-reagin complex and the unknown serum is considered positive.

11-35. In the complement fixation test for syphilis, the serum to be tested is heated to destroy the complement. Hence, the only source of complement is the guinea pig serum. This source of complement must be carefully titrated so that a syphilitic serum will use all of the complement. A weakly positive serum uses only part of the complement, leaving some available to produce partial hemolysis of the sheep cells. The complement fixation test may be performed on serum as well as spinal fluid. A qualitative and a quantitative procedure are outlined in AFM 160-47.

11-36. *Analysis of difficulties.* When difficulties are experienced, they are due, in most instances, to complement. Occasionally, complement is too low in hemolytic activity; this is particularly true in the case of preserved complement. Complement deteriorates rapidly at room temperature, especially when diluted. Complement should always be diluted with cold saline and kept in the refrigerator at all times except when in use.

11-37. Prozone reactions may occur in quantitative tests with complete hemolysis of the serum control and sometimes with incomplete hemolysis of the antigen control. Zonal reactions will also occur if the normal anti-sheep cell agglutinins have not been absorbed from the patient's serum.

11-38. Hemolysin is usually the first reagent suspected in cases of difficulty, but least likely to be a cause of trouble, especially if the reagent has been previously used with success. The unit of anti-sheep hemolysin in the regular tests should be at least 0.5 ml. A few additional causes of error in complement fixation tests may be failure to observe one of the following precautions.

a. Sheep erythrocyte suspension should be kept in the refrigerator when not in use. Always shake before using to secure an even suspension, as the corpuscles settle to the bottom of the container when allowed to stand.

b. Glassware must be chemically clean. Traces of acid, alkali, or detergent may interfere with any serologic test.

c. Pooled nonreactive sera should be pretested to insure nonreactivity with all antigens and freedom from anticomplementary activity.

11-39. *Anticomplementary serums and spinal fluids.* Anticomplementary activity is the nonspecific absorption of complement by tissue extracts and other complex organic materials present in serum and spinal fluid. Occasionally, serums and spinal fluids may be found to be anticomplementary as evidenced by incomplete hemolysis of the controls. In this case, repeat the tests with fresh serum or spinal fluid.

11-40. Occasionally, controls may show incomplete hemolysis due to anticomplementary effects. The unused portion of the serums and of spinal fluids should always be kept in the refrigerator until the test is completed, in case repetitions are required. If difficulties are due to thermostable anticomplementary substance in serums, the serums can usually be satisfactorily tested after preperation by Sach's method, which is outlined in AFM 160-47.

11-41. **Reiter Protein Complement Fixation (RPCF) Test.** The Reiter protein complement fixation test employs a protein extract from the Reiter strain of *T. pallidum* as a source of serologically active antigen. The technique employed in performing the test is the regular complement fixation procedure conducted with one-fifth volumes of reagents. In practice, the RPCF antigen has been relatively simple to produce and quite inexpensive when compared with the antigen of the other true treponemal antigen tests currently in use.

11-42. **Fluorescent Treponemal Antibody Absorption (FTA-ABS) Test.** Earlier in this volume you reviewed some of the principles of fluorescent antibody testing. We will now discuss the FTA-ABS test, which has replaced the FTA-200 test in most laboratories.

11-43. In the FTA-ABS test, the antigen is a stable water soluble extract of *Treponema pallidum* (Nichols strain) which is available commercially. This test can be performed by any laboratory which is equipped to do fluorescent antibody studies. The absorption aspect of the FTA-ABS test is intended to eliminate interference caused by antibodies formed against nonpathogenic *Treponema* (e.g., *T. microdenticum).* Absorption does not eliminate fluorescence with antibodies of yaws, pinta, or related *Treponema*[4].

11-44. In the absorption process, you place a thin smear of *T. pallidum* on a glass slide, allow it to air dry, and fix it in acetone. Next, a dilution of serum to be tested is added to the smear and the smear is incubated. After incubation the slide is washed and fluorescein-tagged AHG is added. An acceptable procedure for the FTA-ABS test is found in the U.S. Public Health Service *Manual of Tests for Syphilis.* The FTA-ABS procedure should be reserved for cases which warrant a test of this level. As with all laboratory tests, this procedure should not be substituted for sound clinical judgment.

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[4] W. E. Deacon, J. B. Lucas, and E. V. Price: Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test for Syphilis. *JAMA*, 198: 624-628, 1966.

MODIFICATIONS

Chapter 5 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.

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Glossary

Absorption—The penetration or taking in of substances into the bulk of a solid or liquid. In blood banking and serology: the taking up of antibodies in a suspension onto the surfaces of particulate antigens.

ACD: Acid-Citrate-Dextrose Solution—An anticoagulant, nutrient, and preservative used in the collection of blood for transfusion.

Achlorhdria—Absence of hydrochloric acid from the gastric secretions.

Acquired Immunity—Immunity resulting from development of active or passive immunity.

Active Immunity—Immunity that results from formation within the body of substances which render a person immune.

Acute Phase—The early stage of an infectious disease in which the symptoms, including elevation of temperature, are manifest. With most infectious diseases this phase includes the first 3 to 5 days after initial onset of illness.

Adsorption—The property of a liquid or solid to retain or concentrate at its surface one or more components (atoms, molecules, or ions) from another solid or liquid. Blood banking and serology: the taking up of antibodies onto the surface of antigen particles.

Agglutination—The collection into clumps of the cells or particles distributed in a fluid.

Agglutination Titer—The highest dilution of a serum that causes clumping of red blood cells.

Agglutinins—Antibodies that cause agglutination or clumping of cells in a suspension.

Agglutinogen—A red blood cell antigen.

AHG—Anti-human globulin (Coombs serum).

Albumin-Reactive Antibodies—Antibodies that fail to give a visible reaction with the corresponding red cells in saline but agglutinate the same cells suspended in bovine albumin.

Allele—One of two or more genes for a given trait at the same locus on homologous chromosomes.

Amboceptor—An immune substance that has an affinity for antigens and complement.

Anamnestic Reaction—An immunological reaction in which antibodies that have previously existed and have disappeared from the blood are rapidly redeveloped on the injection of the original antigen or a different nonspecific antigen.

Anemia—A condition in which the blood is deficient in quantity or quality of erythrocytes.

Anisocytosis—Variation in size of the erythrocytes.

Antibody—A specific substance which is produced in the body in response to the presence of an antigen and which reacts with the antigen in some certain way.

Antibody Combining Site—That particular area or arrangement of atoms on an immune body that determines its specificity.

Anticomplementary Substance—Substance that opposes or counteracts the action of complement.

Antigen—Any substance foreign to the body's antibody-forming tissues which stimulates these tissues to form antibodies.

Antigenic Determinant—Combining site on an antigen.

Antiserum—A serum (liquid portion of blood) containing antibodies.

Antistreptolysin O Titer—The level in the blood or tissues of specific antibodies against the streptolysin O hemolytic factor which is produced by certain streptococci.

Antitoxin—An antibody capable of neutralizing a specific toxin. It is produced in response to the presence of a toxin.

Au^a—Designation for the Auberger antigen.

Autoagglutination—Nonspecific clumping of an individual's cells by factors in his own serum or plasma. It is most common at low temperatures (5°C.).

Autohemagglutinin (Autoagglutinin)—An antibody capable of agglutinating red blood cells in the same individual.

Autoimmune Disease—A disease characterized by the subject's body producing a substance foreign to its antibody-forming tissue, which is stimulated to produce antibodies against the substance.

Azurophilic Granule—Rounded, discrete, reddish-purple granule, smaller than the granules of neutrophiles; 0-10 are common in lymphocytes, and they are very numerous and smaller in the cytoplasm of monocytes.

Beef Erythrocyte Antigen—Substance contained in beef erythrocytes which is capable of absorbing the heterophile antibodies produced as a result of infectious mononucleosis and serum sickness.

Bilirubinemia—The level of the bile pigment bilirubin in blood.

Blocking Antibody—An antibody that coats red cells without promoting agglutination. This coating interferes with the agglutination of the erythrocytes suspended in saline by other antibodies specific for the red cell antigens.

Blood Grouping—Classification of blood specimens into groups (or types) on the basis of the red blood cell antigens.

Blood Group System—A system of related blood group antigens; e.g., the ABO system, the MNS system, the Rh-Hr system, etc.

Bombay Blood Group—The O_h blood group that lacks H-substance.

Buffy Coat—The layer of white blood cells and platelets found overlying the red cells following packing by centrifugation.

Cardiolipin Antigen—Substance composed of extract from fresh beef hearts combined with lecithin and cholesterol. This antigen is used in flocculation and precipitation tests for syphilis.

Cardiolipin Microflocculation—A flocculation test for syphilis involving the reaction of syphilitic reagin with the cardiolipin antigen. The visible reaction appears as the aggregation of antigen particles to form floccules of varying size.

Chancre—A hard, syphilitic, primary ulcer. Usually the first sign of syphilis.

Chromatin—The deeply staining protoplasm of a cell's nucleus.

Chromosome—One of the dark staining, rod-shaped bodies appearing within the nucleus of the cell. It contains the genes.

Closed System—A term used to describe a blood pack or some special type of unit that is sealed under sterile conditions when it is filled, and is not subsequently entered or opened in any way.

Cold Agglutinins—Agglutinins which react optimally at low temperatures.

Comatose—In a coma.

Compatibility Test—A test carried out between serum and erythrocytes to insure that they are not antagonistic. Usually this term refers to the crossmatch between a patient's serum and donor's red cells.

Complement—A complex protein, present in fresh normal blood serum, which participates in various immunologic reactions. It is bound by antigen-antibody aggregates.

Complement Fixation—When antigen unites with its specific antibody, complement, if present, is taken into the combination and becomes inactive or fixed. Its presence or absence as free active complement can be shown by adding sensitized blood cells or blood cells and hemolytic amboceptor to the mixture. If free complement is present, hemolysis will occur: if not, no hemolysis will be observed.

Congenital—Born with a person; existing at or before birth.

Conjugate—In immunofluorescent microscopy, the union of antigen, antibody, or complement with the labeling substance (fluorescein).

Control—A controlled system used to test the correctness of observations. In serological testing, controls are generally set up to check conditions and/or reagents.

Convalescent Phase—That stage of an infectious disease which immediately follows the cessation of clinical symptoms. In most infectious diseases this period is generally considered to be 10 to 14 days after the onset of illness.

C-Reactive Protein—A protein, not normally present in human blood which appears in a wide variety of inflammatory conditions. It is characterized by its ability to react visibly with the C-polysaccharide somatic substance of the pneumococci.

Crenation—The scalloped or notched appearance of the periphery of erythrocytes found when the cells are suspended in a hypertonic solution. Also found on smears and is caused by dirty glassware, slow drying, and poor smearing technique.

Cytolysis—Dissolution of cells by specific antiserum and complement.

Department Of Defense Blood Program—A triservice program established by DOD Directive Number 6480.5, dated 15 May 1962. Under this directive, the Secretary of the Army is assigned certain responsibilities for joint aspects of the Department of Defense Blood Program, to be executed on a joint staff basis, under the direction and control of the Secretary of Defense.

Deoxyribonucleic Acid (DNA)—A substance containing a phosphoric ester of pentose found in the nuclei of all cells.

Determinant—That portion of an antigen molecule which determines the antigen's specificity but alone is not capable of eliciting an immune response.

Dextran—A polymer of dextrose $(C_6H_{12}O_6)_n$ used as a plasma volume expander. It is also sometimes employed as a cell suspending medium for the detection of antibodies.

Differential Heterophil Test—A test designed to differentiate between the three types of heterophil antibodies.

Direct Antiglobulin Test (Direct Coombs Test)—A test for the detection of coated red blood cells (as a result of circulating antibodies) by the use of antihuman globulin.

Dominance—The expressed influence of one gene over another when two or more genes are present. For example, the gene for brown eyes shows dominance over the gene for blue eyes. Blood group genes do not show dominance.

Dosage—Relative expression of gene action. For example, a stronger antibody reaction may be detected from homozygous (e.g., MM) individuals than from heterozygous ones (e.g., MN).

D^u—A variant of the Rho (D) antigen.

Dyscrasia—Abnormality.

Eluate—The antibody containing solution prepared by the elution of red cells.

Elution—The separation of adsorbed antibodies from the surface of red cells by the use of heat, and their placement in solution in the surrounding fluid medium.

Endemic Syphilis—A form of syphilis contracted through direct and indirect contact and limited to certain geographical areas.

Erythroblastosis Fetalis—See Hemolytic Disease of the Newborn.

Erythrocytosis—An increase in the total number of erythrocytes.

Erythropenia—A decrease in the total number of red blood cells in the blood.

Erythropoiesis—The production of erythrocytes.

Etiology—The theory of the causation of a disease.

Exchange Transfusion (Exsanguination Transfusions)—A method in which an infant's blood is withdrawn and simultaneously replaced by compatible blood. It is a means of treating hemolytic disease of the newborn.

Febrile Agglutinins—Agglutinating antibodies produced by the body in response to various fever-producing organisms. Examples are: antibodies directed against the causative agents of typhoid fever, paratyphoid, tularemia, undulant fever, typhus, etc.

Ficin—A proteolytic enzyme obtained from figs.

Flocculation—A phenomenon in which particles dispersed in a medium combine into discrete, usually visible aggregates.

Fluorescein—A red crystalline powder used to tag and identify antibodies in immunofluorescent microscopy.

Fluorescence—Luminescence of a substance when acted upon by short wave radiation. The substance absorbs light of certain wavelengths and emits light of a longer wavelength. The emission terminates simultaneously with the cessation of the incident exciting radiation.

Forssman Antibody—One of the heterophile antibodies which is naturally present in the blood of man in low titer.

Fy^a And Fy^b—Two antigens of the Duffy system.

Gamma Globulin—That fraction of serum globulin which contains most of the antibodies.

Gamete—Male or female reproductive cell.

Ge—Designation for the Gerbich antigen.

Gene—The basic unit of inheritance, which carries one genetically transmissible character and which is arranged on a chromosome.

Genetics—The science of inheritance.

Genotype—The fundamental hereditary or genetic structure of an individual.

Group-Specific Substances—Carbohydrate substances found in the body fluids and tissues of persons whose erythrocytes contain the "A" and "B" antigens which inhibit anti-A and anti-B antibodies.

Guinea Pig Kidney Antigen—A substance used in the differential heterophile test. It absorbs the Forssman and serum sickness antibodies. The infectious mononucleosis antibody is absorbed to a much lesser degree or not at all.

Half-Life—The time required for a radioactive substance to lose half its energy.

Hapten(E)—That portion of an antigenic molecule or antigen complex that determines its immunological specificity. It usually does not stimulate antibody formation by itself, but reacts specifically *in vivo* and *in vitro* with the antibody once it is formed.

Hemagglutination—The agglutination (clumping together) of red blood cells.

Hemagglutination-Inhibition—The prevention of the agglutination of red blood cells.

Hemagglutinin—Any immune substance which causes the agglutination of red blood cells.

Hematoma—A subcutaneous swelling containing effused blood. It can be caused by a traumatic venipuncture.

Hematopoisesis—The formation of red blood cells.

Hemolysin—An antibody capable of causing the lysis or dissolution of blood cells with the consequent release of hemoglobin.

Hemolysis—The lysis (dissolution) of red blood cells.

Hemolytic Anemia—That type of anemia characterized by excessive intravascular destruction of red blood cells.

Hemolytic Disease Of The Newborn (HDN)—A disease manifested in infants as the result of *in utero* incompatibility between antigens of the fetus' red blood cells and maternal antibodies.

Hemostasis—The checking of the flow of blood, especially from a blood vessel.

Heparin—A substance that prevents coagulation of blood by stopping the conversion of prothrombin to thrombin and by neutralizing thrombin.

Heterophile Antibodies—A group of antibodies having an affinity for antigens found in a wide range of organisms. All heterophile antibodies have the common property of agglutinating sheep erythrocytes.

Heterozygous—A genotype composed of two different genes.

Homozygous—A genotype consisting of two identical genes.

H-Substance—A substance that is a precursor of the O gene.

Human Immune (Anti-D) Globulin—An immune serum globulin solution that is injected into a mother just after delivery to prevent Rh isoimmunization and subsequent hemolytic disease of the newborn.

Hypertonic Solution—A solution with an ionic concentration greater than the system with which it is compared.

Hypochromasia—A lack of hemoglobin in red blood cells.

Hypotonic Solution—A solution with an ionic concentration less than the system with which it is compared.

IgG—A synonym for 7-S or γG antibodies.

IgM—Another name for 19-S or γM antibodies.

Immune Bodies—Substances in those afflicted with antigenic disease agents formed by the tissues and capable of destroying or lessening the effect of the disease-producing agent. Also called antibodies.

Immune Response—The response of a body's antibody forming tissue to foreign antigens that results in the formation of antibodies.

Immunity—The state of being resistant to injury, particularly to foreign antigens, and is due to presence in the blood of specific antibodies, such as agglutinins, precipitins, opsoninins, antitoxins, etc.

Immunofluorescence—The technique of tagging antibodies with fluorescent dyes that aid in observing the reaction of these antibodies with specific antigens.

Immunogenic Agent—Any substance capable of inducing immunity or an immune response.

Immunoglobulin—That fraction of serum globulin containing antibodies which has the power to confer immunity.

Immunohematology—The study of the immune response with specific reference to blood.

Immunocompetent Cell—Any cell capable of forming immunoprotein substances (antibodies).

Immunology—The study of resistence to disease.

Immunoprotein—Any protein immune body (antibody) or substance that confers immunity.

Inactivation—The process by which the activity of serum complement is nullified. The usual means is to heat the serum to 56° C. for 30 minutes.

Incompatibility—Agglutination or lysis of cells in the *in vitro* crossmatch procedure before the transfusion of blood.

Incomplete Antibody—An antibody that gives no visible reaction with red cells processing the corresponding specific antigen when these red cells are suspended in saline. The term is somewhat of a misnomer since the antibody itself is complete, but the conditions for its reaction with an antigen are unfavorable.

Infectious Mononucleosis—An acute infectious disease characterized by a sudden onset and acute course, with fever and inflammatory swelling of the lymph nodes, especially those of the cervical region. There is a moderate leukocytosis due almost entirely to abnormal mononuclear cells.

Intragroup Incompatibility—Incompatibility between individuals of the same ABO blood group: the cause of intragroup hemolytic reactions.

In Utero—In the uterus; during fetal life.

In Vitro—Outside of a living thing, i.e., observable in a test tube.

In Vivo—Within the living body.

Isoagglutinin—An antibody capable of only agglutinating cells of some individuals of the same species.

Isoantigens—Antigens found in some members of a species but not in other members.

Isoimmunization (Iso-Sensitization)—An antibody response to antigens from members of the same species.

Isotonic Solution—A solution with the same concentration as the system with which it is compared.

Jk^a and Jk^b—Antigens of the Kidd system.

K, K—Antigens of the Kell system.

Laked—Hemolyzed.

Le^a and Le^b— Designation for the Lewis-a and Lewis-b antigens respectively.

Leukemia—A disease of the blood-forming organs characterized by a marked increase in the number of leukocytes in the blood.

Leukemoid Reaction—A temporary increase in the number of immature leukocytes in the blood associated with a marked increase in the total leukocyte count.

Leukocytosis—An increase in leukocytes in the blood.

Leukopenia—A reduction in the number of leukocytes in the blood.

Linkage—Tendency for two or more genes to be inherited together.

Lipoproteins—A conjugated protein composed of a simple protein and a lipid.

Locus—The position of a gene on a chromosome.

Lu^a and Lu^b—Antigens of the Lutheran system.

Luminescence—Light emission that cannot be attributed to the temperature of the emitting body. It is sometimes characterized as fluorescent and phosphorescent. Phosphorescence is delayed light emission (afterglow), while fluorescence is emission during the period of excitation.

Lymphocytosis—A relative or absolute increase in the number of lymphocytes in the blood.

Lyophilization—The creation of a stable preparation of a biological material by rapid freezing and dehydration of the frozen product under high vacuum.

Lysin—An antibody that has the ability to cause dissolution or lysis of cells.

Lysozyme—A substance present in tears and other body fluids and tissues which destroy bacteria.

Macroglobulin—Any serum globulin with an unusually high molecular weight.

Major Crossmatch—The *in vitro* test between the donor's cells and the patient's serum.

Meiosis—Cell division (reduction division) in which daughter cells have one-half the number of chromosomes of somatic or other body cells. This number is referred to as haploid, abbreviated *n*, and is equal to 24 in humans.

Mendelian Laws—The basic laws of genetic inheritance proposed by Gregor Mendel.

Minor Crossmatch—The *in vitro* test between the patient's cells and the donor's serum.

Mitosis—Ordinary cell division without a reduction in the number of chromosomes. These somatic cells have the diploid number of chromosomes (2n) which is 48 for the human.

National Blood Program—Formerly the title of the Department of Defense-Public Health Service (DOD-PHS) blood program for national emergencies, administered by the Office of Emergency Planning (OEP), formerly the Office of Civil and Defense Mobilization.

National Blood Resource Program—A program established by PHS, HEW, with the support and approval of Congress and administered by NIH. The purpose of the program is to develop ways to reduce wastage of blood and blood products in the United States as a whole.

National Emergency Blood Program—The OEP program superseding the National Blood Program and established by Defense Mobilization Order 8540.2 dated 10 April 1967. The major change is the deletion of the designation of the American National Red Cross as the sole blood collection agency for the Federal agencies.

Natural (Innate) Immunity—Most often, a permanent immunity that results from inherited factors.

Naturally Occurring Antibodies—Those antibodies that occur in a person shortly after birth and throughout adult life but are not produced by injections or pregnancy. They are due to "natural" environmental factors, probably proteins and polysaccharides, taken into the body via the digestive or respiratory systems.

Neutralization—An antigen-antibody reaction in which the reactive effect of a particular antigen is nullified by a specific anitbody.

NIH—National Institute of Health.

Nonsusceptibility—Complete immunity to specific antigenic substances and is due to natural inherent factors.

OEP—Office of Emergency Planning, formerly the Office of Civil and Defense Mobilization.

Open System—A system, such as a blood pack unit, that has been opened or in some way exposed to the atmosphere. The use of transfer packs to withdraw serum from a blood unit renders the unit an open system.

Opsonin—A substance in blood serum which acts upon macroorganisms and other cells, making them more attractive to phagocytes.

Panagglutinable Cells—Red cells that are agglutinated by sera from all adult human beings tested, regardless of the ABO groups.

Panagglutinating Serum—A serum causing the agglutination of all erythrocytes tested, irrespective of their blood group.

Panel of Cells—A series of separate erythrocyte suspensions of known antigenic content used to identify antibodies.

Papain—A proteolytic enzyme obtained from the tropical fruit paw-paw *(Carica papaya).*

Parachromatin—Any of several nonstaining or weakly staining nuclear elements.

Passive Immunity—Immunity produced by injection, absorption, or transfer of preformed antobodies into the subject to be protected.

Pfeiffer Reaction—The lytic destruction of bacteria in the presence of immune serum and complement.

Phagocytin—A bactericidal substance, probably a globulin that can be isolated from polymorphonuclear leukocytes.

Phlebotomy—Withdrawal of blood from a vein.

Phenotype—The visible or physically observable inherited characteristics of an individual, e.g., eye color, blood group, etc.

Plasmapheresis—Return of packed red cells to the donor.

Poikilocytosis—An increased number of abnormally shaped erythrocytes.

Polychromasia—Diffuse basophilia of the erythrocytes.

Polychromatophilia—The presence on a stained blood smear of immature, nonnucleated, bluish staining red blood cells.

Polycythemia—An increase in the total number of red blood cells.

Polypeptide—A complex carbohydrate of high molecular weight found especially as a component of the capsule of various microorganisms.

Polysaccharide—Complex carbohydrates of high molecular weight found especially as a component of the capsule of various microorganisms.

Pooled Cells—A suspension of cells derived from several different donors and then placed together. Pooled cells are used in screening tests for the detection of antibodies.

Postzone Reaction—A weak or irregular antigen-antibody reaction occurring when a great excess of antigen is exposed to a serum containing a relatively low titer of antibody.

Potency Of Antisera—The relative ability of sera to react with the specific antigen. High titer indicates great potency.

Precipitation—An antigen-antibody reaction in which a soluble antigen is caused to settle out by the action of its specific antibody.

Precipitin—An antibody formed in the body in response to soluble antigens. Precipitins react with soluble antigens to form insoluble precipitates or soluble complexes.

Precursor—That which precedes. Erythropoesis: those cells that give rise to immature erythrocytes.

Presumptive Heterophile Test—A test to determine the presence of increased amounts of heterophile antibodies.

Properdin—A natural blood chemical that destroys bacteria and neutralizes viruses.

Prozone Phenomenon—A negative or weak antigen-antibody reaction occurring when serum containing a disproportionately high titer of antibody is exposed to a relatively small quantity of antigen.

Purpura—Small spots on the skin caused by subcutaneous effusion of blood.

Pyknotic Erythrocyte—An immature erythrocyte showing a dense compact nucleus and reduced cytoplasm.

Pyrogenic Reaction—A transfusion response, usually mild, due to pyrogens (q.v.).

Pyrogens—Substances that cause a fever. In blood they are filterable components, probably of bacterial origin that may be present in the anticoagulant solution.

Reagin—An antibodylike substance produced by the body in response to certain types of tissue invasion and destruction. It is found in varying small amounts normally, but is usually increased in syphilis, malaria, and certain other diseases.

Recessiveness—The opposite of dominance (q.v.).

Reticuloendothelial System—Cells of the body with endothelial and reticular qualities that show a common phagocytic behavior toward foreign particles. This group includes endothelial and reticular cells of the spleen, lymph, liver, and bone marrow.

Rh Antibodies (Wiener)—Anti-Rh_0 Anti-rh', Anti-rh'', Anti-Hr_0, Anti-hr', and Anti-hr'' (or Fisher-Race Anti-D, Anti-C, Anti-E, and anti-d (theoretical), anti-c, and anti-e).

Rh_0 Blocking Serum—An Rh_0 antiserum containing antibodies of sufficient high titer and avidity to give a distinct blocking reaction.

Rheumatic Fever—A disease, probably infectious, associated with the presence of hemolytic streptococci in the body. Beginning with an attack of sore throat or pharyngitis, there develops chilliness, rapid rise of temperature, prostration, and painful inflammation of the joints.

Rh Negative Donors—Donors possessing the genotype cde/cde. These donors must be negative for CDE and D^u

Ribonuclear Proteins—A nucleoprotein that yields a ribonucleic acid on hydrolysis.

Rouleaux Formation (Pseudoagglutination)—The formation by human erythrocytes of stacks which appear microscopically as piles of coins. This "stacking" is enhanced by increased fibrinogen and/or globulin levels in the blood.

Secretors—Individuals who possess the "A" and "B" substances in their tissues and secrete it to their saliva, urine, tears, semen, gastric juice, and milk. These secretions are found in about 85 percent of the population.

Serial Dilution—Progressively higher dilutions of a substance arranged in a definite sequence or series.

Serology—The branch of biology which concerns itself with antigens and antibodies and their relationships.

Serum Sickness Antibody—Specific antibody produced in response to a foreign serum, especially if an illness results from the introduction of the foreign serum.

Sex Chromosome—X-chromosomes of a female and Y-chromosomes of a male which carry the genes that determine sex or are sex-linked.

Sorption—The process of taking up and holding either by adsorption or absorption.

Specificity—The special affinity of antigens for their corresponding homologous antibodies.

Spherocyte—A red blood cell which is more spherical, smaller, darker, and more fragile than normal.

Streptolysin O—An oxygen labile, hemolytic factor produced by certain streptococci.

Stroma—The spongy colorless supporting framework of an erythrocyte.

Stromalyzer—Any substance which causes dissolution of a cell's stromatin.

Thrombocytopenia—A decrease in the total number of thrombocytes.
Thrombocytosis—An increase in the number of circulating thrombocytes.
Thrombopoiesis—The formation of thrombocytes.
Thrombosis—Formation of a thrombus or blood clot.
Titer—An expression of the highest dilution of a substance that contains sufficient antibody material to give a visible reaction.
Titration—Determining the quantity of antibody in a serum by means of a serial dilution.
Treponema Pallidum Immobilization Test (TPI)—A test based on the immobilization or death of virulent, motile, spirochetes when they are exposed to the specific antibodies present in syphilitic serum.
Trypsin—A proteolytic enzyme used for the detection of antibodies.
Vasoconstrictor Substances—Substances that cause constriction of blood vessels.
Ve^a—Designation for the Vel antigen.
Venereal Syphilis—A form of syphilis contracted through direct sexual relations.
Warmed Autoantibody—A variety of autoantibodies reacting with equal intensity at body and refrigerator temperatures. Such antibodies do not occur normally, but appear to be produced only by certain individuals who have a remarkable capacity to produce antibodies in general. This may give rise to the disease, acquired hemolytic anemia.
Washed Cells—Cells freed of plasma or serum by repeated centrifugation through fresh volumes of normal saline.
Weil-Felix Reaction—The diagnostic agglutination of Proteus X bacteria by the blood sera of typhus fever cases due to the presence of an antigen in the bacteria common to that found in the causative rickettsial organisms.
Widal Test—A procedure designed to detect antibodies, if present, against the causative organisms of typhoid fever.
X Anthrochromia—A yellow discoloration.
Yt^a—Designation of the Cartwright antigen.

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WORKBOOK

SEROLOGY

SELF-TEACHING AIDS
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This workbook places the materials you need *where* you need them while you are studying. In it, you will find the Study Reference Guide, the Chapter Review Exercises and their answers, and the Volume Review Exercise. You can easily compare textual references with chapter exercise items without flipping pages back and forth in your text. You will not misplace any one of these essential study materials. You will have a single reference pamphlet in the proper sequence for learning.

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STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume. Use the Guide for review before you take the closed-book Course Examination.

2. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the items missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Numbers 300 through 309

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300	Introduction; Mechanics of Immunity; Antigen - Antibody Interaction; pages 1-9
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CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 1

Objectives: To understand the mechanics of the immune response and its role in the production of antibodies; to be able to explain antigen-antibody reactions and the part they play in common serological techniques.

1. What degree of immunity would you expect if a person's body makes a feeble immune response to an infection? (1-1)

2. From the given definition for immunology, why does a good general health level determine a person's ability to resist infection? (1-2)

3. Are all bacteria and viruses immunogenic agents? (1-2)

4. Where are antibodies most likely to be found? (1-2)

5. Define serology. (1-2)

6. List some of the substances other than microorganisms which may be antigenic. (1-3)

7. Why don't humans suffer from foot-and-mouth disease in the same manner as cows? (1-4)

8. List four factors that play a role in determing one's innate immunity. (1-5)

9. What type of immunity does a nursing baby receive from its mother? (1-6)

10. People who had typhoid fever once usually are protected against reinfection by what type of immunity? (1-6, 7)

11. Do all infections result in immunity to reinfection? (1-7)

12. Is protection in active acquired immunity either absent or complete? (1-8)

13. How are antitoxins prepared? (1-9)

14. How does active acquired immunity differ from passive acquired immunity? (1-7-9)

15. Why is passive immunity of short duration? (1-10)

16. Give a simple definition for antigen that will cover any kind of substance. (2-2)

17. Are antigens always proteins? (2-3)

18. List several antigenic substances other than protein. (2-3,4)

19. When we say "foreign to the body," what do we mean? (2-5,6)

20. What is an autoimmune disease? (2-6)

21. Are antibodies always proteins? (2-7)

22. Give three synonyms for antibody. (2-7)

23. What is a precipitin? (2-8)

24. List three types of human globulin. (2-9)

25. Name the most common property shared by antigens and antibodies. (2-5-10)

26. In ultracentrifugation techniques, how are immunoglobulins separated? (2-10)

27. Which immunoglobulin has the smallest molecule? (2-13)

28. Name two types of cells extensively involved in antibody synthesis. (2-14)

29. What is an immunocompetent cell? (2-14)

30. Which theory of antibody production maintains that there is a "mirror image" mold which produces antibody? (2-15)

31. What is the usual significance of lysis in a serological test? (2-16,17)

32. Why are guinea pigs used as a source of complement for serological tests? (2-18)

33. What part does complement play in antigen-antibody reactions? (2-19)

34. List three ways in which bacteria in the presence of complement is affected by an immune serum. (2-20)

35. Define opsonization. (2-20)

36. What kind of substance is complement? (2-21)

37. The "lock and key" concept has been used to describe which feature of antigens and antibodies? (2-23)

38. Are antigen-antibody reactions reversible? (2-24)

39. What kind of reaction does specificity imply? (2-25)

40. Are cross-reacting antigens and antibodies always a disadvantage in a serological procedure? (2-26,27)

41. What is the basic difference in making dilutions in hemagglutination procedures and in precipitin procedures? (2-28)

42. What type of reactions will occur if too much antigen is present in a test? (2-28)

43. What is the term that specifically expresses the reciprocal of a dilution? (3-2)

44. Give two functions of cell suspensions used in serological procedures. (3-4)

45. Write the formula for finding packed cell volume (PCV). (3-5)

46. What will be the total volume if you have 0.45 ml. of packed cells and wish to make a 5-percent suspension? (3-11)

47. What does serial dilution mean? (3-13)

48. What kind of instrument is used in a Takatsy microtitration to make the dilutions? (3-14)

49. Write the formula for finding dilution. (3-16)

50. List four common methods for identifying antibodies. (3-24)

51. What two techniques are combined in immunoelectrophoresis? (3-24)

52. Define fluorescence. (3-26)

53. How do eyepiece filters affect colors seen through fluorescent microscopes? (3-27)

54. What types of objectives give the best results in fluorescent microscopy? (3-28)

55. Can dark-field condensers be used with fluorescent microscopes? (3-29)

56. How does increasing the numerical aperture of the objective affect images through the fluorescent microscope? (3-31)

57. What type of light source is recommended for fluorescent work? (3-32)

58. Which filter removes scattered excitation light from the viewer's vision? (3-33)

59. Which filter removes autofluorescence? (3-35)

60. Why isn't serum labeling as desirable as globulin labeling? (3-36)

61. What dye is currently recommended as a labeling agent? (3-37)

62. How is labeling quality estimated? (3-38)

63. What percentage of a reduction in antibody titer might you expect as a result of conjugating a globulin with fluorescein? (3-39)

64. How are unwanted antibodies removed from globulin solutions? (3-40)

65. What effect does sorption have on labeled globulins? (3-42)

66. To what types of antigen-antibody reactions is fluorescent staining limited? (3-43)

67. How do we reduce the complications due to natural antibodies in fluorescent tests? (3-44)

68. List four methods of labeling used in fluorescent studies. (3-45-51)

69. Before fluorescent studies can be reported, what pretest do you have to run on the conjugate? (3-52)

CHAPTER 2

Objectives: To learn how to distinguish heterophile antibodies from nonheterophile antibodies; to show a knowledge of heterophile tests; and to understand basic antigen-antibody reactions that result in agglutination and to apply these phenomena to serological testing procedures.

1. Why has infectious mononucleosis (IM) been called a kind of "controlled leukemia"? (4-1)

2. Distinguish isophile antibodies from heterophile antibodies. (4-3)

3. Why do people develop Forssman antibodies? (4-5)

4. What is Forssman antigen? (4-5-7)

5. Name the three best known heterophile systems. (4-7)

6. What is the usual human source of antigen that stimulates serum sickness antibodies? (4-8)

7. What type of antibody is the IM antibody? (4-9)

8. What type of antibody is the anti-sheep hemolysin found in low titer in the serum of IM infected patients? (4-9)

9. Where is IM antibody produced? (4-10)

10. What is the most likely cause of infectious mononucleosis? (4-13)

11. Contrast IM immune response in babies with IM immune response in young adults. (4-14)

12. What characteristic of infectious mononucleosis antibody permits the use of sheep cells to indicate reaction? (4-15)

13. Is the Paul-Bunnell test a diagnostic (identifying) test? (4-15,16)

14. In Davidsohn's differential test for heterophile antibodies, which antibodies are absorbed and removed from the specimen? (4-18, 19)

15. Name several of the antigens used in slide tests for infectious mononucleosis. (4-19-28)

16. What permits slide test procedures to omit an adsorption step? (4-19-28)

17. Which of the various antigen/indicator systems has the most affinity for IM antibodies? (4-28)

18. Why is typhoid fever called a febrile disease? (5-2)

19. What are febrile agglutination tests? (5-2)

20. What is an anamnestic reaction? (5-3)

21. Why must saline be used in the Widal test? (5-6)

22. Why should care be exercised when reading flagellar antigen-antibody reactions? (5-8)

23. What kind of febrile agglutination test results would you expect on patients previously immunized for typhoid fever? (5-9)

24. Does the Weil-Felix test detect anti-*Proteus* antibodies? (5-11)

25. What are the advantages of slide test procedures? Disadvantages? (5-14)

26. What is thermal amplitude? (6-3)

27. What is the most likely cause of primary atypical pneumonia (PAP)? (6-5, 6)

28. List three serological procedures useful in diagnosing PAP. (6-1-7)

CHAPTER 3

Objectives: To understand the latex-fixation reactions as used in detecting rheumatoid arthritis and related diseases; to show a knowledge of the precipitin reaction and methods of using precipitation in test procedures; and to understand the antistreptolysin test.

1. What is the substance produced by the body in rheumatoid arthritis? (7-2)

2. What type of immune response does rheumatoid arthritis cause? (7-2)

3. What are autoimmune diseases? (7-2)

4. What is the sheep cell coating antigen used in the Rose test? (7-4)

5. Is the antigen in latex-fixation tests the latex particles? (7-5, 6)

6. What other diseases besides rheumatoid arthritis result in the production of abnormal macroglobulins that cause the latex fixation to be positive? (7-8)

7. Why are controls especially mandatory when you perform slide tests using latex and other preserved antigens? (7-9)

8. Are anti-C reactive protein antibodies detected in tests for C-reactive protein? (7-11)

9. Does the preciptin reaction always result in a visible precipitate being formed? (8-1)

10. Which reaction is more sensitive, agglutination or precipitation? How do we know this? (8-2)

11. In what group of tests is the precipitin reaction most frequently employed? (8-3)

12. Why is simple diffusion called "simple"? (8-4)

13. What type of diffusion is used in the Ouchterlony technique? (8-6)

14. What organisms produce streptolysin O? (9-1)

15. Streptolysin is composed of two components, streptolysin O and S. Why don't we test for streptolysin S? (9-1)

16. How do antibiotics affect antistreptolysin test titers? (9-6)

17. What is the most frequent source of error in the antistreptolysin test? (9-7)

CHAPTER 4

Objective: To understand the principles, methodology, and quality control of serological tests for syphilis.

1. Who were the discoverers of the organism that causes syphilis? (Intro.-1)

2. What was the name that was first given to the syphilis organism? (Intro.-1)

3. Who developed the first effective treatment for syphilis? (Intro.-2)

4. What was the first effective medication for treating syphilis? (Intro.-2)

5. When did syphilis first appear? (10-2)

6. What are the two forms of syphilis? (10-5)

7. What is usually the first symptom of a venereal syphilis infection? (10-6)

8. List four stages of syphilis. (10-6)

9. Why is it unlikely that a person will contract venereal syphilis other than through sexual intercourse? (10-7)

10. What kind of immune response does a syphilis infection elicit? (10-8)

11. How does transmission of endemic syphilis differ from venereal syphilis? (10-9)

12. Name three serologically related diseases that give false positive syphilis tests. (10-10)

13. What are the two organisms that cause rat-bite fever? (10-13)

14. Is reagin an anti-*Treponema* antibody? (10-14)

15. Which *Treponema* organisms are used to produce antigens specific for syphilis? (10-14)

16. Is antibody response to a syphilis infection detectable in the first few weeks after exposure? (10-15)

17. Would a specimen that gives a positive test in the VDRL test be positive in all other kinds of tests for syphilis? (10-16)

18. How should qualitative flocculation tests be reported? (11-1)

19. Should quantitative tests be reported as weakly reactive? (11-1)

20. Why should serum controls of graded reactivity be tested before being put into use? (11-4, 5)

21. How often should you check water baths used in serological procedures for syphilis? (11-7)

22. How should you hold a square-tipped antigen dispensing needle? (11-11)

23. How should you hold a beveled antigen dispensing needle? (11-11)

24. What test must you run on any needle used for antigen delivery? (11-11)

25. What is the recommended cleaner for tubes, slides, etc., to be used in syphilis tests? (11-13)

26. Are syphilis tests valid if you use a new batch of antigen that is much more potent than that you previously used? (11-15)

27. Why is Merthiolate® the recommended preservative for syphilis serology specimens? (11-20)

28. What is the antigen used in the cardiolipin microflocculation test (VDRL) composed of? (11-21)

29. Does a quantitative test report of a very high titer mean the patient has an unusually severe syphilis infection? (11-27-29)

30. In prozone reactions, how do strongly positive specimens react? (11-30)

31. Name four tests that use a cardiolipin antigen for detecting reagin. (11-31-33)

32. What does hemolysis in the complement fixation test indicate? (11-34)

33. Why do we heat a specimen to destroy complement and turn around and add complement to the test? (11-35)

34. Which reagent is the least likely source of trouble in complement fixation tests? (11-38)

35. What does the expression "anticomplementary specimen" mean? (11-39)

36. What is the source of the protein extract used as an antigen in the Reiter protein complement- fixation tests? (11-41)

37. The FTA-ABS fluorescent procedures allow reduced interference from which group or organisms? (11-43)

MODIFICATIONS

Pages 13- 16 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.

ANSWERS FOR CHAPTER REVIEW EXERCISES

CHAPTER 1

1. Slight or no immunity.

2. Ability to resist infection is determined by all factors that affect the body. A good general health level means that the person would be less susceptible to any type of foreign agent than an unhealthy person.

3. No. A microorganism is an immunogenic agent *only* if capable of causing an immune response which results in the production of antibodies.

4. In the blood. They are not there at all times or immediately after exposure to an immunogenic agent, but they appear there at some point in the immune response.

5. The study of sera. This is accomplished through laboratory tests and procedures which study the immune response.

6. Plant pollens, proteins, polysaccharides, and lipids.

7. Human beings possess an innate or natural resistance to this disease whereas cows do not.

8. Ethnic group, age, sex, diet, etc.

9. Acquired immunity.

10. Active acquired immunity.

11. No. Infection by some organisms gives little or no immunity to reinfection.

12. No. Immunity may be graded into several levels between none and complete immunity.

13. Suspensions of microorganisms are injected into rabbits, horses, etc., and serum is collected after the animal has formed antibodies againist the foreign substance.

14. In active acquired immunity antibodies have been produced as a result of a previous infection. In passive acquired immunity the antibodies are gained from an outside source such as an injection.

15. When passive immunity is gained through performed antibodies, the person receiving them does not undergo immune response as a result of receiving such immunity. Consequently, as soon as these transferred antibodies are removed from the blood, the passive immunity will no longer exist.

16. Any substance which elicits an immune response.

17. No. Antigens do not necessarily have to be proteins. Many nonprotein substances are antigenic.

18. Polysaccharides, glycopeptides, polypeptides, nucleic acids, cardiolipin, etc.

19. "Foreign to the body" means the substance in question is not native to the body. In order to account for autoimmune response, this expression should convey a meaning of "foreign to the body's antibody forming tissues."

20. An autoimmune disease is one in which a tissue destroying process produces abnormal substances (macroglobulins) which elicit an immune response in the patient producing the abnormal substance.

21. Yes. Most antibodies are found as serum proteins; however, antibodies also occur in body fluids other than blood serum.

22. Antiserum, immunoprotein, immunoglobulin.

23. A precipitin is an antibody that reacts with soluble antigen.

24. Alpha (α), beta (β), and gamma (γ).

25. Antigens and antibodies are both high molecular weight substances.

26. According to sedimentation constant, which is a code for differentiating them according to molecular weight.

27. IgG. This immunoglobulin can cross the placenta, while IgA and IgM cannot.

28. Lymphocytes and plasmacytes.

29. A cell that is capable of forming antibody.

30. The direct template theory.

31. In most instances, lysis indicates that a complement-fixing reaction has taken place.

32. Guinea pig complement is of high lytic quality and uniformity. Complement from several guinea pigs is blended to produce that used for testing.

33. When complement takes part in antigen-antibody reactions, it becomes bound or fixed.

34. Lethal action, lytic action, and opsonization.

35. Action of opsonins in making cells or bacteria more susceptible to phagocytosis.

36. Complement is composed of globulin and of globulinlike serum.

37. Complementarity.

38. Yes. Reactions may be reversed. In reactions in which binding forces are weak, reactions may be reversed or unbound.

39. It implies that the reaction is between components that posses particular properties that allow them to react. We refer to these special properties as complementarity.

40. No. Cross reactions are useful in ideniifying certain antigens or antibodies.

41. In hemagglutination procedures, the amount of antibody used is varied (diluted), where as in precipitin tests the amount of antigen is varied.

42. A postzone reaction.

43. Titer.

44. Cell suspensions may be a source of antigen, an indicator system, or both.

45. Packed cell volume = $\frac{\text{total volume} \times \text{\% solution desired}}{100}$.

46. 9 ml.

47. Sequential reduction of concentration in mathematical progression.

48. Calibrated loop.

49. Dilution = $\frac{\text{total volume in tube}}{\text{volume of serum in tube}}$.

50. Complement-fixation, hemagglutination-inhibition, precipitin, neutralization, etc.

51. Immunodiffusion and electrophoresis.

52. Luminescene of a substance caused by absorption of light of a certain wavelength and simultaneous emission of light of a longer wavelength.

53. The filters determine the color seen.

54. Apochromatic or fluorite.

55. Yes.

56. Increasing the numerical aperature increases image brightness.

57. Mercury arc.

58. The primary filter.

59. The secondary filter.

60. Because the label is attached to nonantibody as well as antibody and this is a waste of the labeling agent.

61. Fluorescein isothiocyanate.

62. By the fluorescein/protein ratio (F/P).

63. Less than 50 percent reduction in titer.

64. By sorption (absorption).

65. It makes them more specific.

66. Theoretically, fluorescent staining can be used in any antigen-antibody reaction.

67. By using proper controls.

68. Direct, indirect, inhibition, and complement staining.

69. The conjugate must be tested for specificity.

CHAPTER 2

1. Infectious mononucleosis usually causes an elevated white blood cell count, sometimes quite high. The most striking feature of this disease is without a doubt the wide assortment of atypical lymphocytes seen on blood smears from IM patients. These atypical lymphs often resemble the abnormal cells seen in lymphocytic leukemia.

2. An isophile antibody is one that reacts only with certain members of a species. For example, the anti-A antibody found in blood reacts only with those persons carrying A antigen. A heterophile antibody is one that reacts with an antigen that is common to all members of a species. For example, an anti-sheep cell antibody reacts with the blood cells of all sheep. A heterophile antibody of human origin will react with a heterophile antigen of horses, sheep, or any other animal possessing a competent antigen.

3. People comes into contact with Forssman antigens through inhalation, ingestion, or infection. They respond to the presence of these antigens and produce Forssman antibodies.

4. Forssman antigen is a heterophile antigen. It is found on human A and AB cells, sheep erythrocytes, and in many different strains of bacteria.

5. Forssman, serum sickness, and infectious mononucleosis.

6. Serum from horses used to produce antitoxin is the usual source of the serum sickness heterophile antigen. Horse serum is a potent protein solution, and the immune response to it is very strong in certain persons.

7. An agglutinin. The serum of IM infected patients also contains an anti-sheep hemolysin which is not observed in presumptive tests because complement is not present in the specimen.

8. The anti-sheep hemolysin in the serum of IM infected patients is a naturally occuring Forssman antibody.

9. Studies indicate that IM antibody is formed in lymphoid tissue.

10. Epstein-Barr virus.

11. Babies show very little immune response as measured by heterophile titers. Young adults very often exhibit good response and very high heterophile titers.

12. Infectious mononucleosis antibody is an anti-sheep cell agglutinin.

13. No. This test is referred to as a presumptive heterophile test because it doesn't discriminate the three kinds of heterophile antibodies.

14. Serum sickness and Forssman antibodies

15. Stabilized sheep cells, treated sheep cells, and preserved horse cells.

16. The antigen/indicators used in slide test procedures have a greater affinity for IM antibodies than for Forssman or serum sickness antibodies. This makes an adsorption step unnecessary.

17. Horse cells.

18. Typhoid fever produces a characteristic fever.

19. Tests that detect antiagglutinins produced in response to organisms causing infections that characteristically result in particular fever patterns.

20. An incidental immune response to a previous immunizing agent.

21. To make the mixture electrolytic.

22. The flagellar antigen-antibody reaction is soft and fluffy. It is easily broken up by vigorous shaking.

23. Little or no reaction for 0 antigen and positive for H antigen.

24. No. The antigen used in this test is a *Proteus* antigen. The test detects antibodies produced in response to rickettsial diseases.

25. Slide tests can usually be performed faster and more economical to perform than tube tests. The most important disadvantage is that some specificity and accuracy is sacrificed in most slide test procedures.

26. The temperature range in which antigens and antibodies react.

27. *Mycoplasma pneumoniae.*

28. Three serological tests used in diagnosing PAP are the cold agglutination test, the *Streptococcus MG* test, and the fluroescent antibody test.

CHAPTER 3

1. A high molecular weight macroglobulin.

2. An autoimmune response.

3. Diseases that cause the body to produce abnormal substances which in turn elicit an immune response resulting in antibodies against the substance.

4. Anti-sheep globulin.

5. No. The antigen is normal human gamma globulin. The latex particles act as a carrier for this antigen.

6. Collagen and tissue destruction diseases, such as lupus erythematosus, liver diseases, hypergammaglobulinemia, etc.

7. Most slide test antigens such as latex antigens are preserved antigens and are stored in the refrigerator, sometimes for a long period of time. This allows them to become lumpy. The technician must use controls so he can compare known positive and negative reactions.

8. No. In the C-reactive protein test we detect C-reactive protein, an abnormal macroglobulin that precipitates C-polysaccharide of certain pneumococcal organisms. The macroglobulin is not classed as an antibody.

9. No. Sometimes the reaction results in the formation of soluble complexes of antigen and antibody. Usually some other step such as neutralization, inhibition, or tagging is required to show that a reaction has taken place.

10. The agglutination reaction. We know this because strong agglutination of a particular antigen occurs with a smaller quantity of antiserum than precipitation of soluble antigens.

11. Syphilis serologies.

12. In simple diffusion only one component (the antigen) diffuses or moves. The antiserum remains stationary.

13. Double diffusion.

14. Certain *Streptococcus* strains, mostly Group A.

15. Because it is nonantigenic, and no practical procedure is available for detecting it.

16. Penicillin, aureomycin, and certain other antibiotics inhibit the in vivo production of streptolysin O. The treated patient does not develop as strong an antibody response as the untreated patient.

17. Inactivated or decomposed streptolysin O antigen.

CHAPTER 4

1. Schaudinn and Hoffman.

2. *Spirochaeta pallida.*

3. Paul Ehrlich.

4. Compound 606 (Salvarsan).

5. Approximately 500 years ago.

6. Venereal and endemic syphilis.

7. A primary lesion or chancre.

8. Primary, secondary, latent, and tertiary.

9. Indirect contact of syphilis is unlikely because the organism dies readily under unfavorable environmental conditions.

10. None or very little.

11. It is spread by direct and indirect contact.

12. Yaws, pinta, and rat-bite fever.

13. *Streptobacillus moniliformis* and *Spirocheta morsus muris.*

14. No. Reagin is an antibodylike, high molecular weight substance produced by the body in syphilis and several other tissue destroying diseases. It is not a specific anti-*Treponema* antibody.

15. Living and killed Nichols strain and avirulent Reiter strain.

16. No. Antibody response is not detectable in the first few weeks of infection. Antibodies can usually be detected in from 1 to 3 weeks after the appearance of the primary lesion or chancre. The primary lesion may not appear until the third or fourth week of infection.

17. No. The different syphilis procedures test for different antibodies or antibodylike substances. Therefore, a positive by one method does not necessarily mean another method will yield positive results.

18. Reactive (R), weakly-reactive (W), and nonreactive (N).

19. No. Quantitative tests are only performed on those specimens that are positive in the qualitative test. Therefore, quantitative tests can only be reported as reactive. Titer in these tests is taken as the last dilution to give a definite reactive test result.

20. The testing of serum controls of graded reactivity provides uniform patterns of reactivity. This is the only way possible to standardize what is reported as a positive or a negative.

21. Each time they are used during the day.

22. It should be held perpendicular to the tabletop.

23. The beveled dispensing needle should be held at a slight angle.

24. The needle must be calibrated.

25. Sulfuric acid-dichromate cleaning solution.

26. No. Antigens and other reagents must be of uniform reactivity.

27. Merthiolate®, chemically, does not interfere with the test, nor does it introduce a significant dilution factor. The amount required for bacteriostatic action is very small.

28. Cholesterol and an alcoholic mixture of cardiolipids and lecithin.

29. No. There is no correlation between the severity of the disease and the amount of reagin in a single specimen. There is significance, however, if the patient shows a rise in titer on repeat specimens.

30. Weakly.

31. The CMF or VDRL, the rapid plasma reagin test, the plasma crit test and the complement fixation test.

MODIFICATIONS

Page 25 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.

STOP–

1. **MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.**
2. **USE NUMBER 1 PENCIL.**

90413 03 22

VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp #1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a #1 black lead pencil.

NOTE: TEXT PAGE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Text Page Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Text Pages* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed.* Go to the VRE booklet and locate the *Text Page Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (001) The body's ability to protect itself against disease-producing agents is known as

a. sensitization.
b. immunology.
c. immunity.
d. immunization.

2. (001) Which of the following is *least* likely to cause an inmune response?

a. Pollen.
b. Bacteria.
c. Viruses.
d. Rickettsia.

3. (002) The body's response to an infection produces

a. passive acquired immunity.
b. active acquired immunity.
c. natural resistance.
d. native immunity.

4. (002) Passive immunity lasts

a. forever.
b. several years.
c. 1 year.
d. a short time.

5. (003) All of the following statements concerning antigens are true *except* that antigens

a. stimulate the formation of *specific* antibodies.
b. are usually formed by the globulin fraction of serum protein.
c. usually have molecular weights of 10,000 or more.
d. are known as immunogenic agents.

6. (003) The polysaccharide of pneumococcal organisms stimulates

a. a single type of anitbody.
b. several types of antibodies.
c. as many as 80 different types of antibodies.
d. several hundred types of antibodies.

7. (003) Antibodies that make soluble foreign proteins insoluble are known as

a. precipitins.
b. agglutinins.
c. reagins.
d. neutralizing antibodies.

8. (004) The Greek characters α, β, and γ are used to identify fractions of

a. serum.
b. blood.
c. globulin.
d. albumin.

9. (005) Structurally, immunoglobulins consist of

a. nucleic acid.
b. clones of mesenchymall cells.
c. polysaccharide aggregates.
d. polypeptide chains.

10. (005) Which of the following is a differentiating characteristic of the immunoglobulin fraction IgG?

a. IgG occurs in large amounts in tears.
b. IgG is produced rapidly after immunization but dimishes as a strong IgM response takes over.
c. IgG molecules are small enough to cross the human placenta.
d. IgG occurs in internal secretions and may provide protection where other types do not.

11. (005-006) A cell that produces antibodies is described as being

a. immunogenic.
b. immunoglobulin.
c. immunocompetent.
d. an immune body.

12. (006) In which template theory of antibody production is the genetic memory of a cell affected by the presence of antigen within the cell?

a. The direct template theory.
b. The indirect template theory.
c. The natural template theory.
d. All of the above theories.

13. (006) Complement may be described as

a. an immunogenic agent.
b. an immunoprotein.
c. a lytic substance.
d. a neutralizing antibody.

14. (006) In the Pfeiffer reaction, complement was found to be

a. oxygen stabile.
b. oxygen labile.
c. heat stabile.
d. heat labile.

15. (006-009) Complement-fixation is the reaction of

a. antigen and complement only.
b. antibody and complement only.
c. antigen, complement, and antibody.
d. bacteria and complement.

16. (009) For reaction to take place, the antigen and antibody must be

a. similar.
b. far apart.
c. dissimilar.
d. brought close together.

17. (009) In a serological reaction, the zone of antigen excess is known as the

a. equivalence zone.
b. optimum zone.
c. prezone.
d. postzone.

18. (009) In a postzone reaction, a false negative result is due to

a. an excess of antigen.
b. diluted antigen.
c. an excess of antibody.
d. diluted antibody.

19. (010) Selection of particular types of indicator cells for use in serological tests is determined by

a. the antibodies they carry only.
b. the antigens they carry only.
c. both antibodies and antigens.
d. neither antibodies nor antigens.

20. (010) What volume of packed cells is required to make 70 ml of a 2-percent cell suspension?

a. 0.14.
b. 0.7.
c. 1.4.
d. 14.

21. (011) The microtitration technique requires a Plexiglas sheet, dropping pipettes, and a

a. wire loop.
b. calibrated loop.
c. micro-loop.
d. plastic loop.

22. (011) The loop used in the microtitration techniques is cleaned with

a. alcohol.
b. hot soapy water.
c. a flame.
d. saline solution.

23. (011) You are calculating a dilution and have a tube in which 0.0014 ml of serum is diluted to a total volume of 1 ml. The dilution of the serum would be

a. 1:714.
b. 1:514.
c. 1:314.
d. 1:114.

24. (012) Complement-fixation tests are used to detect fixation of complement by

a. globulins.
b. antigens.
c. immunogenic protein.
d. antibodies.

25. (012-013) Which of the following is *not* a method of detecting antibodies?

a. Precipitation.
b. Centrifugation.
c. Gel diffusion.
d. Hemagglutination.

26. (013) Changing eyepiece filters in a fluorescent microscope causes a change in

a. color.
b. clarity.
c. specimen emission.
d. specimen absorption.

27. (013) What portion of the spectrum is most suitable for fluorescent microscopy examinations of fluorescein-stained smears?

a. Red-yellow.
b. Ultraviolet-blue.
c. Blue-green.
d. Ultraviolet-orange.

28. (013) The function of the secondary filter in a fluorescent microscope is to

a. remove stray light.
b. mainly protect the eyes.
c. improve image brightness.
d. give the image characteristic wavelengths.

29. (014) The dye of choice for fluorescent studies is fluorescein

a. chloride.
b. cyanide.
c. isocyanate.
d. isothiocyanate.

30. (014) Sorption (absorption) of globulin and serum results in the

a. removal of unwanted bacteria.
b. reduction of unwanted color.
c. reduction of nonspecific staining.
d. removal of conjugates.

31. (014-015) In the multistage fluorescent antibody (FA) [illegible] observable results start with the formation of

a. precipitates.
b. clumps.
c. colors.
d. lattices.

32. (015) When using the direct fluorescent antibody (FA) staining technique to identify unknown antigen, what staining agent should you use?

a. Labeled antibody.
b. Unlabeled antibody.
c. Fluorescein.
d. Antiglobulin.

33. (016) In the inhibition method of fluorescent antibody (FA) staining, the smear is *first* exposed to

a. labeled antigen.
b. labeled antibody.
c. unlabeled antigen.
d. unlabeled antibody.

34. (016) In the indirect fluorescent antibody (FA) staning method, unlabeled antibody is both

a. labeled antibody and smears.
b. labeled antigen and smears.
c. antibody and autoglobulin.
d. antigen and antibody.

35. (017) Before fluorescent antibody tests can be reported, the conjugate must be tested for

a. antigenicity.
b. specificity.
c. homoglucity.
d complementarity.

36. (018) Which statement concerning infectious mononucleosis is *true?*

a. Infectious mononucleosis rarely kills anyone.
b. Infectious mononucleosis frequently cripples those infected with it.
c. Infectious mononucleosis causes a great many deaths.
d. Infectious mononucleosis is more prevalent among older people.

37. (018-019) Studies have implicated atypical lymphocytes in infectious mononucleosis (IM) by showing that these cells produce immunologic

a. macroglobulins.
b. antiglobulins.
c. heterophile antigens.
d. IM antigens.

38. (019) Forssman antibodies develop mainly from exposure to

a. horse serum.
b. plants and bacteria.
c. serum porteins.
d. isophile antigens.

39. (019) Three types of heterophile antibodies are

a. antiglobulins, antitoxin, and infectious mononucleosis.
b. infectious mononucleosis, isophile, and Forssman.
c. Forssman, isophile, and serum sickness.
d. serum sickness, Forssman, and infectious mononucleosis.

40. (020) Studies have implicated which of the following as a possible cause of infectious mononucleosis?

a. Epstein-Barr virus.
b. Bacterial organism.
c. *Listeria monocytogenes.*
d. Malignant lymphoma.

41. (020-021) A patient who has *not* received an injection of horse serum is considered to have IM if the presumptive titer is

a. 28.
b. 56.
c. 112.
d. 224.

42. (020-021) During the second to fourth week of atypical infectious mononucleosis infection, the patient's titer is usually

a. lowest.
b. variable.
c. highest.
d. not detectable.

43. (020) We call the Paul-Bunnel test a presumptive test because if the titer is 1:224 or higher, we assume which of the following are present in the serum?

a. Infectious mononucleosis antibodies.
b. Anti-sheep cell hemolysins.
c. Serum sickness antibodies.
d. Forssman antibodies.

44. (023) In serological tests, slide test procedures requires which of the following?

a. Increased antigen.
b. Increased antibody.
c. Adequate controls.
d. Complement inactivation.

45. (025) Compared to sheep cell tests, horse cells give

a. higher titers.
b. lower titers.
c. variable titers.
d. unrelated titers.

46. (025) In an anamnestic reaction, antibody-producing tissue is restimulated to produce antibodies against previous

a. immunizations.
b. viral infections.
c. bacterial infections.
d. sensitizing antibodies.

47. (025) The bacterial antigen used in the original Widal test was

a. live and motile *S. typhi.*
b. live and motile *Salmonella* sp.
c. killed *Salmonella* sp.
d. lypholized *S. typhi.*

48. (026) The medium in which antigen-antibody reactions occur must be

a. warmed.
b. refrigerated.
c. buffered.
d. electrolytic.

49. (026-027) The *Proteus* "O" antigens are

a. somatic antigens.
b. anti-typhus antigens.
c. rickettsial agglutinins.
d. nonspecific.

50. (027) The Weil-Felix test is considered diagnostic if

a. the titer is 1:80.
b. reaction is with a single strain of *Proteus.*
c. there is a rise in titer.
d. no performed antibodies are present.

51. (027) Compared to tube tests, slide tests for febrile agglutinins are

a. less accurate.
b. more accurate.
c. more diagnostic.
d. more specific.

52. (027) The range in which temperature dependent antibodies will react is known as thermal

a. variation.
b. amplitude.
c. latitude.
d. agglutination range.

53. (027-028) The cold agglutination test is considered diagnostic of primary atypical pneumonia if the titer increases

a. slightly.
b. moderately.
c. twofold.
d. fourfold.

54. (029-030) Latex-fixation tests for rheumatoid arthritis detect

a. anti-latex antibodies.
b. rheumatoid antigens.
c. autoimmune antibodies.
d. polystyrene-reactive antibodies.

55. (029) The major deficiency of the sheep cell agglutination and Rose tests for rheumatoid arthritis (RA) is their lack of

a. observable agglutination.
b. specificity.
c. reactivity.
d. simplicity.

56. (030) Polystyrene latex particles in RA tests are coated with

a. macroglobulin.
b. gamma globulin.
c. anti-gamma globulin.
d. anti-rheumatoid arthritis antibodies.

57. (030) Because stored antigen may become lumpy, it is best to

a. use slide test screening.
b. run control tests on it.
c. run known positive tests on it.
d. dilute it with saline.

58. (031) Soluble antigen-antibody reactions can occur in

a. agglutination reactions.
b. precipitin reactions.
c. hemagglutination-inhibition reactions.
d. hemagglutination reactions.

59. (031) In simple diffusion tests, where does reaction occur?

a. At the interface.
b. In the antigen.
c. In the antiserum.
d. throughout the media.

60. (032) A source of error in the ASO test is

a. residual antigen.
b. inactivated antigen.
c. antibiotic treatment.
d. excess complement.

61. (034) It is believed that syphilis first appeared as a disease of man

a. 65 years ago.
b. 300 years ago.
c. 500 years ago.
d. 1000 years ago.

62. (035) The first sign of a syphilis infection is called the

a. primary lesion.
b. initial lesion.
c. primary eruption.
d. initial sore.

63. (035) Venereal syphilis differs from endemic syphilis in geographical occurrence and

a. organs infected.
b. causative agent.
c. antibody response.
d. mode of transmission.

64. (036) The antigens used to detect reagin are

a. lipid complexes.
b. treponemal antigens.
c. Nichols antigens.
d. Reiter antigens.

65. (036) After the appearance of a chancre in primary syphilis, antibody response is usually detectable

a. immediately.
b. within 1 week.
c. not later than 2 weeks.
d. within 1 to 3 weeks.

66. (036-037) A single high titer on a patient suspected of having syphilis suggests that the patient

a. may or may not have the disease.
b. has inactive syphilis.
c. has chancres.
d. has active syphilis.

67. (037) Control specimens are used in standard syphilis tests to reproduce an established

a. limit of reactivity.
b. reactivity pattern.
c. negative reaction.
d. antibody dilution.

68. (038) Water baths used in standard test for syphilis should be checked

a. once a day.
b. each time used.
c. weekly.
d. twice weekly.

69. (039) Syphilitic serums are heated at 56° C. for 30 minutes to

a. increase reactivity.
b. inactivate the complement.
c. inactivate nonspecific antigens.
d. activate reagin.

70. (039-040) Reagin appears in the blood of a syphilitic patient about how many weeks after infection?

a. 1 week.
b. 2 weeks.
c. 5 weeks.
d. 6 weeks.

71. (040) Which of the following sources of error may be eliminated by performing titers on all positive screening tests?

a. Mixing half quantities.
b. Chemically inpure reagents.
c. Prozone reactions.
d. Faulty inactivation.

MODIFICATIONS

Page 37 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.